
BACKGROUND: Cruciferous vegetables protect against prostate cancer. Indole-3-carbinol (I3C) and its major metabolite 3,3'-diindolylmethane (DIM), exhibit antitumor activities in vitro and in vivo. Several synthetic ring-substituted dihaloDIMs (ring-DIMs) appear to have increased anticancer activity. METHODS: Inhibition of LNCaP prostate cancer cell growth was measured by a WST-1 cell viability assay. Cytoplasmic and nuclear proteins were analyzed by immunoblotting and immunofluorescence. Androgen receptor (AR) activation was assessed by measuring prostate-specific antigen (PSA) expression and using LNCaP cells containing human AR and an AR-dependent probasin promoter-green fluorescent protein (GFP) construct. RESULTS: Like DIM, several ring-substituted dihaloDIM analogs, namely 4,4'-dibromo-, 4,4'-dichloro-, 7,7'-dibromo-, and 7,7'-dichloroDIM, significantly inhibited DHT-stimulated growth of LNCaP cells at concentrations >/=1 microM. We observed structure-dependent differences for the effects of the ring-DIMs on AR expression, nuclear AR accumulation and PSA levels in LNCaP cells after 24 hr. Both 4,4'- and 7,7'-dibromoDIM decreased AR protein and mRNA levels, whereas 4,4'- and 7,7'-dichloroDIM had minimal effect. All four dihaloDIMs (10 and 30 microM) significantly decreased PSA protein and mRNA levels. Immunofluorescence studies showed that only the dibromoDIMs increased nuclear localization of AR. All ring-DIMs caused a concentration-dependent decrease in fluorescence induced by the synthetic androgen R1881 in LNCaP cells transfected with wild-type human AR and an androgen-responsive probasin promoter-GFP gene construct, with potencies up to 10-fold greater than that of DIM. CONCLUSION: The antiandrogenic effects of ring-DIMs suggest they may form the basis for the development of novel agents against hormone-sensitive prostate cancer, alone or in combination with other drugs.


The intake of arginine aspartate has been shown to increase anabolic hormones like human growth hormone (hGH) and glucagon. The aim of our study was to investigate whether daily intake of two different dosages of arginine aspartate during four weeks affects selected parameters of overtraining syndrome like performance, metabolic and endocrine parameters. Thirty male endurance-trained athletes were included in a randomized, double-blind, placebo-controlled study and divided into three groups. During four weeks, they ingested either arginine aspartate with a high concentration (H) of 5.7 g arginine and 8.7 g aspartate, with a low concentration (L) of 2.8 g arginine and 2.2 g aspartate or placebo
(P). VO(2)peak and time to exhaustion were determined on a cycling ergometer in an incremental exercise test before and after supplementation. Before and after each incremental exercise test, concentrations of hGH, glucagon, testosterone, cortisol, ferritine, lactate, and urea were measured. Compared to placebo, no significant differences on endurance performance (VO(2)peak, time to exhaustion), endocrine (concentration of hGH, glucagon, cortisol, and testosterone) and metabolic parameters (concentration of lactate, ferritine, and urea) were found after chronic arginine aspartate supplementation. The chronic intake of arginine asparate during four weeks by male endurance athletes showed independent of dosage no influence on performance, selected metabolic or endocrine parameters. Consequently, there seems to be no apparent reason why the supplementation of arginine aspartate should be an effective ergogenic aid. The practice of using arginine aspartate as potential ergogens should be critically reevaluated. Further investigations with higher dosage and extended supplementation periods should be performed.


Newborn male and female rat pups were injected with either 2 mg or 4 mg monosodium aspartate (MSA)/g body weight or diluent on alternate days for the first 9 days of life. Both doses of the amino acid had profound effects on the sexually dimorphic growth hormone secretory profiles in adulthood. There were no measurable levels of growth hormone in any of the plasma samples obtained during 8 continuous hr of serial blood collections from the adult males and females treated neonatally with 4 mg of MSA. Male rats treated with half the dose of the amino acid (i.e., 2 mg MSA/g) exhibited typical masculine profiles of growth hormone release, except that the amplitudes of the ultradian pulses were reduced to 10-20% of normal male levels. Otherwise, like normal males, the peaks occurred about every 3-4 hr and the intervening 2.5-hr troughs had undetectable levels of growth hormone. In a similar sense, females treated with 2 mg of MSA maintained their sexually dimorphic pattern of plasma growth hormone, i.e., frequent pulses of hormone followed by short-lived troughs. However, the peaks rarely exceeded 20 ng/ml and the troughs usually fell to a measurable 8 to 10 ng/ml resulting in an approximate 75% reduction in the mean plasma concentration. Growth hormone- and gender-dependent expression of CYP2C7, 2C11, 2C12, 2C13, 2A1, 2A2, and 3A2 (mRNAs, proteins, and catalytic activities) were generally unaffected by neonatal exposure to 2 mg of MSA. In contrast, the higher 4-mg dose of the amino acid completely or near completely suppressed male-specific CYP2C11, 2C13, 2A2, and 3A2 expression while inducing small increases in female-specific CYP2C12 and female-predominant CYP2A1 in the treated males. Females exposed to the 4 mg MSA dose exhibited less severe isoform changes characterized by small reductions in CYP2C12 and 2C7 levels. Whereas expression levels of most of the CYP isoforms in both sexes were lowest in the pubertal (47-day-old) rats, and occasionally higher in the adults (207-day-old) rats, the effects of neonatal MSA were the same at all ages studied. Since each of the CYP isoforms are regulated by different "signaling elements" in the sexually dimorphic plasma growth hormone profiles, it is possible to correlate MSA-induced alterations in CYP expression levels to specific changes in the gender-dependent growth hormone profiles.

Ahmad, A., W. A. Sakr, et al. "Novel targets for detection of cancer and their modulation by
Cancer affects the lives of millions of people. Several signaling pathways have been proposed as therapeutic targets for cancer therapy, and many more continue to be validated. With the identification and validation of therapeutic targets comes the question of designing novel strategies to effectively counter such targets. Natural compounds from dietary sources form the basis of many ancient medicinal systems. They are pleiotropic i.e. they act on multiple targets, and, therefore, are often the first agents to be tested against a novel therapeutic target. This review article summarizes the knowledge so far on some actively pursued targets - Notch, CXCR4, Wnt and sonic hedgehog (shh) pathways, the process of epithelial-mesenchymal transition (EMT) as well as molecular markers such as uPA-uPAR, survivin, FoxM1, and the microRNAs. We have performed an extensive survey of literature to list modulation of these targets by natural agents such as curcumin, indole-3-carbinol (I3C), 3,3'-diindolylmethane (DIM), resveratrol, epigallocatechin-3-gallate (EGCG), genistein etc. We believe that this review will stimulate further research for elucidating and appreciating the value of these wonderful gifts from nature.


The objectives of this study were to determine whether stress attenuates the pituitary LH response to excitatory amino acids by altering expression of glutamate receptor 1 (GluR1) and N-methyl-D-aspartic acid (NMDA) receptor mRNA levels in the hypothalamus or pituitary, and assess whether stress influences testicular levels of mRNA or protein for steroidogenic enzymes. Three hours (h) of immobilization stress was associated with a greater than 7-fold increase in serum corticosterone, and a marked reduction in serum testosterone (T) concentrations. Stress did not significantly alter hypothalamic or pituitary GluR1 and NMDA receptor mRNA levels. Although transcript levels for P450SCC and P45017alpha mRNA in the testis were unchanged in stressed rats, western blotting of testicular fractions revealed reduced amounts of P450SCC and 3beta-HSD, but not P45017alpha. The data suggest that immobilization stress reduces T production by suppressing the translation of transcripts for P450SCC and 3beta-HSD, but the attenuated LH response of stressed animals to NMDA is not mediated by altered hypothalamic or pituitary expression of GluR1 and NMDA receptor levels.


Inactivation of survival pathways such as NF-kappaB, cyclooxygenase (COX-2), or epidermal growth factor receptor (EGFR) signaling individually may not be sufficient for the treatment of advanced pancreatic cancer (PC) as suggested by recent clinical trials. 3,3'-Diindolylmethane (B-DIM) is an inhibitor of NF-kappaB and COX-2 and is a well-known chemopreventive agent. We hypothesized that the inhibition of NF-kappaB and COX-2 by B-DIM concurrently with the inhibition of EGFR by erlotinib will potentiate the anti-tumor effects of cytotoxic drug gemcitabine, which has been tested both in vitro and in vivo.
Inhibition of viable cells in seven PC cell lines treated with B-DIM, erlotinib, or gemcitabine alone or their combinations was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Significant inhibition in cell viability was observed in PC cells expressing high levels of COX-2, EGFR, and NF-kappaB proteins. The observed inhibition was associated with an increase in apoptosis as assessed by ELISA. A significant down-regulation in the expression of COX-2, NF-kappaB, and EGFR in BxPC-3, COLO-357, and HPAC cells was observed, suggesting that simultaneous targeting of EGFR, NF-kappaB, and COX-2 is more effective than targeting either signaling pathway separately. Our in vitro results were further supported by in vivo studies showing that B-DIM in combination with erlotinib and gemcitabine was significantly more effective than individual agents. Based on our preclinical in vitro and in vivo results, we conclude that this multi-targeted combination could be developed for the treatment of PC patients whose tumors express high levels of COX-2, EGFR, and NF-kappaB.


The first activating mutation of the FSH receptor (FSHR*D567G) was identified in a gonadotropin-deficient hypophysectomized man who exhibited persistent spermatogenesis and fertility with only androgen replacement. We have determined the ability of FSHR* activity to maintain spermatogenesis and/or steroidogenesis during gonadotropin and androgen deprivation in mature transgenic FSHR* mice (Tg(Abpa-FSHR*D567G)1Cmal), hereafter referred to as Tg-FSHR* mice. Testes of untreated adult Tg-FSHR* males were equivalent in weight to nontransgenic controls but exhibited increased total Sertoli cell (24%) and spermatogonia (34%) numbers and nonsignificantly elevated spermatocyte-spermatid numbers (13%-17%). During sustained GNRH1 agonist treatment that markedly reduced (96%-98%) serum LH and testosterone (T) and decreased serum FSH (68%-72%), the testes of GNRH1 agonist-treated Tg-FSHR* mice remained significantly larger than treated nontransgenic controls. After 4 wk of gonadotropin suppression, Sertoli cell numbers were reduced in Tg-FSHR* testes to levels comparable with nontransgenic testes, whereas spermatogonia numbers were maintained at higher levels relative to nontransgenic testes. However, after 8 wk of GNRH1 agonist treatment, the total spermatogonia, spermatocyte, or postmeiotic spermatid numbers were reduced to equivalent levels in Tg-FSHR* and nontransgenic mice. FSHR* effects were further examined in gonadotropin-deficient hypogonadal Gnrh1hpG/Gnrh1hpG (Gnrh1(-/-)) mice during testicular regression following withdrawal of T after maximal T-stimulated spermatogenesis. After 6 wk of T withdrawal, spermatogonia, spermatocyte, and postmeiotic spermatid numbers in Tg-FSHR* Gnrh1(-/-) testes decreased to levels found in untreated Tg-FSHR* Gnrh1(-/-) testes. Basal serum T levels in untreated Tg-FSHR* Gnrh1(-/-) males were 2-fold higher than Gnrh1(-/-) controls, but following T treatment/withdrawal, serum T and epididymal weights declined to basal levels found in nontransgenic Gnrh1(-/-) mice. Therefore, FSHR* was unable to sustain circulating T or androgen-dependent epididymal size or postmeiotic spermatogenic development. We conclude that FSHR* activity enhances Sertoli and spermatogenic development in normal testes but has limited ability to maintain spermatogenesis during gonadotropin deficiency, in which the testicular response provided by the FSHR*D567G mutation resembled typical FSH-mediated but not steroidogenic activity.

Angwafor, F., 3rd and M. L. Anderson (2008). "An open label, dose response study to determine the effect of a dietary supplement on dihydrotestosterone, testosterone and estradiol levels in
BACKGROUND: Maintaining endogenous testosterone (T) levels as men age may slow the symptoms of sarcopenia, andropause and decline in physical performance. Drugs inhibiting the enzyme 5alpha-reductase (5AR) produce increased blood levels of T and decreased levels of dihydrotestosterone (DHT). However, symptoms of gynecomastia have been reported due to the aromatase (AER) enzyme converting excess T to estradiol (ES). The carotenoid astaxanthin (AX) from Haematococcus pluvialis, Saw Palmetto berry lipid extract (SPLE) from Serenoa repens and the precise combination of these dietary supplements, Alphastat(R) (Mytosterone(trade mark)), have been reported to have inhibitory effects on both 5AR and AER in-vitro. Concomitant regulation of both enzymes in-vivo would cause DHT and ES blood levels to decrease and T levels to increase. The purpose of this clinical study was to determine if patented Alphastat(R) (Mytosterone(trade mark)) could produce these effects in a dose dependent manner.

METHODS: To investigate this clinically, 42 healthy males ages 37 to 70 years were divided into two groups of twenty-one and dosed with either 800 mg/day or 2000 mg/day of Alphastat(R) (Mytosterone(trade mark)) for fourteen days. Blood samples were collected on days 0, 3, 7 and 14 and assayed for T, DHT and ES. Body weight and blood pressure data were collected prior to blood collection. One-way, repeated measures analysis of variance (ANOVA-RM) was performed at a significance level of alpha = 0.05 to determine differences from baseline within each group. Two-way analysis of variance (ANOVA-2) was performed after baseline subtraction, at a significance level of alpha = 0.05 to determine differences between dose groups. Results are expressed as means +/- SEM. RESULTS: ANOVA-RM showed significant within group increases in serum total T and significant decreases in serum DHT from baseline in both dose groups at a significance level of alpha = 0.05. Significant decreases in serum ES are reported for the 2000 mg/day dose group and not the 800 mg/day dose group. Significant within group effects were confirmed using ANOVA-2 analyses after baseline subtraction. ANOVA-2 analyses also showed no significant difference between dose groups with regard to the increase of T or the decrease of DHT. It did show a significant dose dependant decrease in serum ES levels. CONCLUSION: Both dose groups showed significant (p = 0.05) increases in T and decreases in DHT within three days of treatment with Alphastat(R) (Mytosterone(trade mark)). Between group statistical analysis showed no significant (p = 0.05) difference, indicating the effect was not dose dependent and that 800 mg/per day is equally effective as 2000 mg/day for increasing T and lowering DHT. Blood levels of ES however, decreased significantly (p = 0.05) in the 2000 mg/day dose group but not in the 800 mg/day dose group indicating a dose dependant decrease in E levels.


A double-blind parallel group comparison design clinical study was conducted in Japanese patients with mild to moderate erectile dysfunction to investigate the efficacy of a supplement containing Pycnogenolo(R) and L-arginine. Subjects were instructed to take a supplement (Pycnogenolo(R) 60 mg/day, L-arginine 690 mg/day and aspartic acid 552 mg/day) or an identical placebo for 8 weeks, and the results were assessed using the five-item erectile domain (IIEF-5) of the International Index of Erectile Function. Additionally, blood biochemistry, urinalysis and salivary testosterone were measured. Eight weeks of supplement intake improved the total score of the IIEF-5. In particular, a marked improvement was observed in 'hardness of erection' and 'satisfaction with sexual intercourse'. A decrease in blood pressure, aspartate transaminase and gamma-glutamyl transpeptidase (gamma-GTP), and a slight increase in
salivary testosterone were observed in the supplement group. No adverse reactions were observed during the study period. In conclusion, Pycnogenol(R) in combination with L-arginine as a dietary supplement is effective and safe in Japanese patients with mild to moderate erectile dysfunction.


N-methyl-D,L-aspartic acid (NMA), an agonist of the neurotransmitter glutamate has been shown to acutely stimulate the release of prolactin (PRL) in intact rats and monkeys. To further investigate the role of neuroexcitatory amino acids in PRL secretion, the effects of NMA administration were examined on PRL release in long term orchidectomized adult rhesus monkeys, in both the absence and presence of testosterone. Intact and long term castrated adult male monkeys weighing between 8-13 kg, were implanted with a catheter via the saphenous vein for blood withdrawal and drug infusion. Blood samples were collected at 10 min intervals for 50 min before and 70 min after administration of the drug or vehicle. Plasma PRL concentrations were estimated using radioimmunoassay. Whereas a single iv injection of NMA (15 mg/kg BW) induced a prompt discharge of PRL in intact monkeys, an identical dose had surprisingly no effect on PRL secretion in orchidectomized animals. On the other hand, plasma PRL increases in response to a challenge dose of thyrotropin releasing hormone (TRH; 6 micrograms/kg BW, iv) were similar in magnitude in the two groups of monkeys. Testosterone replacement in orchidectomized animals by parenteral administration of testosterone enanthate (200 mg/wk) reinitiated the PRL responsiveness to acute NMA stimulation. These results indicate that N-methyl-D-aspartic acid (NMDA) dependent drive to PRL release in the adult male rhesus monkey may be overtly influenced by the sex steroid milieu.

The present study investigated the role of D-aspartic acid (D-Asp) in ovarian steroidogenesis and its effect on aromatase activity in the lizard, Podarcis s. sicula. It was determined that D-Asp concentrations vary significantly during phases of the reproductive cycle: they vary inversely with testosterone concentrations and directly with oestradiol concentrations in the ovary and plasma. Experimental treatment showed that administration of D-Asp induces a decrease in testosterone and an increase in oestradiol, and that treatment with other amino acids (L-Asp, D-Glu and D-Ala) instead of D-Asp has no effects. Experiments in vitro confirmed these results. Furthermore, these experiments showed an increase in aromatase activity, as the addition of D-Asp either to fresh ovarian tissue homogenate or to acetonic powder of ovarian follicles induced a significant increase in the conversion of testosterone to oestradiol. Aromatase activity is four times greater in the presence of D-Asp than in its absence. However, almost equivalent values of the two K(m) values (both approximately 25 nmol l(-1)) indicate that aromatase has the same catalytic properties in both cases.


Medical treatments have become available for benign hypertrophy of the prostate, including alpha-receptor blocking agents and S-alpha-reductase inhibitors. Drugs derived from plants, for which no
precise mechanism of action has been described, are widely used for this purpose in Europe. In a randomised, double-blind, placebo-controlled multicentre study, 200 patients (recruited between April and October 1993) with symptomatic benign prostatic hyperplasia were treated with either 20 mg beta-sitosterol (which contains a mixture of phytosterols) three times per day or placebo. Primary end-point was a difference of modified Boyarsky score between treatment groups after 6 months; secondary end-points were changes in International Prostate Symptom Score (IPSS), urine flow, and prostate volume. Modified Boyarsky score decreased significantly with a mean of -6.7 (SD 4.0) points in the beta-sitosterol-treated group versus -2.1 (3.2) points in the placebo group p < 0.01. There was a decrease in IPSS (-7.4 [3.8] points in the beta-sitosterol-treated group vs -2.1 [3.8] points in the placebo group) and changes in urine flow parameters: beta-sitosterol treatment resulted in increasing peak flow (15.2 [5.7] mL/s from 9.9 [2.5] mL/s), and decrease of mean residual urinary volume (30.4 [39.9] mL from 65.8 [20.8] mL). These parameters did not change in the placebo group (p < 0.01). No relevant reduction of prostatic volume was observed in either group. Significant improvement in symptoms and urinary flow parameters show the effectiveness of beta-sitosterol in the treatment of benign prostatic hyperplasia.


Formation of bile acids from sitosterol in bile-fistulated female Wistar rats was studied with use of 4-14C-labeled sitosterol and sitosterol labeled with 3H in specific positions. The major part (about 75%) of the 14C radioactivity recovered as bile acids in bile after intravenous administration of [4-14C]sitosterol was found to be considerably more polar than cholic acid, and only trace amounts of radioactivity had chromatographic properties similar to those of cholic acid and chenodeoxycholic acid. It was shown that polar metabolites were formed by intermediate oxidation of the 3 beta-hydroxyl group (loss of 3H from 3 alpha-3H-labeled sitosterol) and that the most polar fraction did not contain a hydroxyl group at C7 (retention of 3H in 7 alpha,7 beta-3H2-labeled sitosterol). Furthermore, the polar metabolites had lost at least the terminal 6 or 7 carbon atoms of the side chain (loss of 3H from 22,23-3H2- and 24,28-3H2-labeled sitosterol). Experiments with 3H-labeled 7 alpha-hydroxysitosterol and 4-14C-labeled 26-hydroxysitosterol showed that none of these compounds was an efficient precursor to the polar metabolites. By analysis of purified most polar products of [4-14C] sitosterol by radio-gas chromatography and the same products of 7 alpha,7 beta-[2H2]sitosterol by combined gas chromatography-mass spectrometry, two major metabolites could be identified as C21 bile acids. One metabolite had three hydroxyl groups (3 alpha, 15, and unknown), and one had two hydroxyl groups (3 alpha, 15) and one keto group. Considerably less C21 bile acids were formed from [4-14C]sitosterol in male than in female Wistar rats. The C21 bile acids formed in male rats did not contain a 15-hydroxyl group. Conversion of a [4-14C]sitosterol into C21 bile acids did also occur in adrenalectomized and ovariectomized rats, indicating that endocrine tissues are not involved. Experiments with isolated perfused liver gave direct evidence that the overall conversion of sitosterol into C21 bile acids occurs in this organ. Intravenously injected 7 alpha,7 beta-3H-labeled campesterol gave a product pattern identical to that of 4-14C-labeled sitosterol. Possible mechanisms for hepatic conversion of sitosterol and campesterol into C21 bile acids are discussed.

Tamoxifen (TXF; an antiestrogen), cyproterone acetate (CYP; an antiandrogen) and mifepristone (MIF; an antigestagen) did not affect kindling parameters (afterdischarge threshold, seizure severity, seizure duration and afterdischarge duration) in fully-kindled rats. TXF (50 mg/kg) and CYP (50 mg/kg), when combined with carbamazepine, or phenobarbital, both antiepileptics administered at their highest subprotective doses of 15 mg/kg, resulted in significant reduction of the seizure and afterdischarge durations, both in male and female rats. Additionally, the combination of carbamazepine and cyproterone markedly increased the afterdischarge threshold in fully-kindled rats of both genders. The interaction between antihormones and carbamazepine, or phenobarbital, was not reversed by the respective gonadal hormones (estradiol, progesterone, and testosterone), kainic acid, or strychnine. However, the TXF-, and CYP-induced effect on the action of carbamazepine was abolished by bicuculline, N-methyl-D-aspartic acid and aminophylline. The effect of TXF on the protective activity of phenobarbital was reversed by bicuculline and N-methyl-D-aspartic acid. Finally, the CYP-mediated effect on phenobarbital action was abolished by bicuculline and aminophylline. Neither TXF nor CYP altered free plasma levels and brain levels of carbamazepine or phenobarbital, so a pharmacokinetic interaction between antihormones and antiepileptic drugs is not probable. In view of the present data, it may be suggested that the protective activity of the antiestrogen and antiandrogen are mostly associated with the enhancement of GABA-ergic and purinergic transmission in the central nervous system. Also the augmentation of glutamatergic transmission, realized through NMDA receptors, may be involved in the mechanism of antiseizure action of TXF and CYP.

The aim of this study was to evaluate the efficacy of three antihormones, tamoxifen (TXF, an antiestrogen), mifepristone (MIF, an antiprogesterone) and cyproterone (CYP, an antiandrogen) in two major models of experimental epilepsy, electrically and pentetrazole (PTZ)-evoked seizures in mice. TXF (20-50 mg/kg) significantly raised the threshold for electroconvulsions in female mice, whereas CYP was active in male mice. Similar effects were observed in castrated mice. Different data were obtained in sexually immature animals since both TXF and CYP exerted anticonvulsive effects in animals of both genders. MIF (5-50 mg/kg) remained without effect on electrically evoked seizures in mice. The anticonvulsive action of TXF was reversed by aminophylline, bicuculline, kainic acid and N-methyl-D-aspartic acid, but not by estradiol or strychnine. The protective action of CYP was reversed by aminophylline and bicuculline, but not by testosterone, kainic acid, N-methyl-D-aspartic acid or strychnine. All three antihormones were ineffective against PTZ-induced convulsions in mice. Our results suggest that the action of TXF and CYP might be indirectly associated with the respective hormonal receptor-mediated events, but the nature of this dependence is unclear and further investigations are needed to elucidate this phenomenon.

The present results refer to the action of three gonadal steroid antihormones, tamoxifen (TXF, an...
estrogen antagonist), cyproterone acetate (CYP, an antiandrogen) and mifepristone (MIF, a progesterone antagonist) on seizure phenomena in mice. TXF and CYP at their lowest protective dose in the electroconvulsive threshold test, enhanced the antiseizure efficacy of some antiepileptic drugs. TXF (20 mg/kg) potentiated the protective activity of valproate, diphenylhydantoin and clonazepam, but not that of carbamazepine or phenobarbital, against maximal electroshock-induced convulsions in female mice. CYP (40 mg/kg) enhanced the anticonvulsant action of valproate, carbamazepine, diphenylhydantoin and clonazepam, but not that of phenobarbital, against maximal electroshock in male animals. MIF failed to affect the electroconvulsive threshold or the efficacy of antiepileptic drugs in maximal electroshock. The effect of TXF or CYP upon the electroconvulsive threshold and on the action of antiepileptics was not reversed by sex steroid hormones (estradiol, testosterone, progesterone). However, the TXF-induced elevation of the electroconvulsive threshold was abolished by bicuculline, N-methyl-D-aspartic acid and kainic acid, and partially reversed by aminophylline, strychnine being ineffective in this respect. The action of CYP on the threshold for electroconvulsions was partially reversed by bicuculline and aminophylline. Both glutamatergic agonists and strychnine remained ineffective in this respect. Moreover, the action of TXF or CYP on the activity of antiepileptics was not influenced by strychnine, and reversed to various extents by the remaining convulsants. In contrast to maximal electroshock, none of the three antihormones affected the protective action of antiepileptic drugs against pentylenetetrazol-induced seizures in mice. Neither TXF nor CYP altered the free plasma levels of antiepileptic drugs, so a pharmacokinetic interaction is not probable. The combined treatment of the two antihormones with antiepileptic drugs, providing 50% protection against maximal electroshock, did not affect motor performance in mice, and did not result in significant long-term memory deficits. Our data indicate that steroid receptor-mediated events may be indirectly associated with seizure phenomena in the central nervous system and can modulate the protective activity of some conventional antiepileptic drugs.


The effects of androgen precursors, combined with herbal extracts designed to enhance testosterone formation and reduce conversion of androgens to estrogens was studied in young men. Subjects performed 3 days of resistance training per week for 8 weeks. Each day during Weeks 1, 2, 4, 5, 7, and 8, subjects consumed either placebo (PL; n = 10) or a supplement (ANDRO-6; n = 10), which contained daily doses of 300 mg androstenedione, 150 mg DHEA, 750 mg Tribulus terrestris, 625 mg Chrysin, 300 mg Indole-3-carbinol, and 540 mg Saw palmetto. Serum androstenedione concentrations were higher in ANDRO-6 after 2, 5, and 8 weeks (p <.05), while serum estrone concentrations of free and total testosterone were unchanged in both groups. Estradiol was elevated at weeks 2, 5, and 8 of andro-6 (p = .05), muscle strength increased similarly from 0 to 4% again treatment the acute effect one third daily dose studied in 10 men 23 years of age androstenedione was 150 or 360 mg min after ingestion or these data provide evidence that addition herbal extracts does not result reduce estrogenic augment adaptations resistance training blockquote

Previous studies have provided evidence that D-Asp plays a role in steroid-mediated reproductive biology in amphibians, reptiles, birds and mammals. To examine the molecular involvement of D-Asp on steroidogenic pathway regulation, we analysed the expression of StAR, P450 aromatase and 5alphaRed2 mRNAs in Pelophylax esculentus testis, either in relation to the reproductive cycle or D-Asp treatment. Basal StAR mRNA levels, as well as D-Asp and testosterone concentrations, were higher in reproductive than in post-reproductive frogs. D-Asp treatment increased StAR mRNA expression and immunolocalisation in both the reproductive and post-reproductive periods. In control testis, aromatase mRNA levels were higher in the post-reproductive period, but following D-Asp administration, they increased only in the reproductive period. The level of 5alphaRed2 mRNA was higher in reproductive frogs than in post-reproductive frogs, and it increased after D-Asp treatment only in the post-reproductive phase. Our results suggest that, in P. esculentus testis, D-Asp increases StAR mRNA in both periods, and P450 aromatase and 5alphaRed2 mRNAs at different points during the reproductive cycle.


d-Aspartic acid is an endogenous amino acid occurring in the endocrine glands as well as in the nervous system of various animal phyla. Our previous studies have provided evidence that d-aspartate plays a role in the induction of estradiol synthesis in gonads. Recently, we have also demonstrated that d-aspartic acid induces P450 aromatase mRNA expression in the frog (Pelophylax esculentus) testis. P450 aromatase is the key enzyme in the estrogen synthetic pathway and irreversibly converts testosterone into 17beta-estradiol. In this study, we firstly investigated the immunolocalisation of P450 aromatase in the brain of P. esculentus, which has never previously been described in amphibians. Therefore, to test the hypothesis that d-aspartate mediates a local synthesis of P450 aromatase in the frog brain, we administered d-aspartate in vivo to male frogs and then assessed brain aromatase expression, sex hormone levels and sex hormone receptor expression. We found that d-aspartate enhances brain aromatase expression (mRNA and protein) through the CREB pathway. Then, P450 aromatase induces 17beta-estradiol production from testosterone, with a consequent increase of its receptor. Therefore, the regulation of d-aspartate-mediated P450 aromatase expression could be an important step in the control of neuroendocrine regulation of the reproductive axis. Accordingly, we found that the sites of P450 aromatase immunoreactivity in the frog brain correspond to the areas known to be involved in neurosteroid synthesis.


The inducing effects of some flavonoids (flavone, flavanone, tangeretin and quercetin) and model substances have been studied in rats, and the activity and the expression of drug-metabolizing enzymes have been compared in rats. The addition of flavonoids to the diet (0.3% w/w) for 2 weeks did not change the liver cytochrome P450 content nor the activities of the NADPH-cytochrome P450 and NADH-cytochrome b5 reductases, but it affected the activities of phase I and phase II enzymes. Flavone, and to a
lesser extent tangeretin, increased the activities mediated by the P450 1A1,2 (EROD) and 2B1,2 (PROD) as well as the activities of p-nitrophenol UDP-glucuronyl transferase (UGT) and glutathione transferase (GST). Flavanone mainly enhanced PROD, UGT and GST, whereas quercetin did not modify any enzyme activities. None of the tested flavonoids modulated the activities catalyzed by P450 2E1, 3A and 4A. Immunoblotting studies showed that flavone and tangeretin increased the expression of cytochrome P450 1A and 2B forms, whereas flavanone only induced cytochrome P450 2B. Flavone and to a lesser extent flavanone, markedly increased the phenol-UGT protein level. Both flavone and flavanone also increased the androsterone- and testosterone-UGTs, whereas tangeretin and quercetin did not increase any UGT isoform. We concluded that the flavonoids tested specifically affected the expression of the drug-metabolizing isozymes in rat liver, their inducing properties were dependent on their chemical structures.


Inactivating mutations of the luteinizing hormone receptor (LHR) gene in males induce Leydig cell agenesis or hypoplasia, while activating mutations cause testotoxicosis. Recently, it was demonstrated that a somatic heterozygous activating mutation of the LHR gene (Asp578His), limited to the tumor, was the cause of Leydig cell adenomas in three unrelated patients. We describe the molecular study of two unrelated boys with gonadotropin-independent hypersecretion of testosterone due to Leydig cell adenomas. Genomic DNA was extracted from the tumor, the adjacent normal testis tissue, and blood leukocytes. Both individuals exhibited an heterozygous missense mutation, limited only to the tumor, consisting of a guanine (G) to cytosine (C) substitution at codon 578 (GAT to CAT), turning aspartic acid into histidine. The presence of the same mutation in different ethnic groups demonstrates the existence of a mutational hot spot in the LHR gene. Indeed, this mutation occurs at the conserved aspartic acid residue at amino acid 578, where a substitution by glycine is the most common mutation observed in testotoxicosis and where a substitution by tyrosine has been linked to a more severe clinical phenotype where diffuse Leydig cell hyperplasia is found. Our results confirm the fact that somatic activating mutations of gonadotropin receptors are involved in gonadal tumorigenesis.


3,3'-Diindolylmethane (DIM) is a major in vivo derivative of indole-3-carbinol, which is present in cruciferous vegetables and has been reported to possess anti-carcinogenic properties. In the present study, we examined whether DIM inhibits the development of prostate cancer using the transgenic adenocarcinoma mouse prostate (TRAMP) model. DIM feeding inhibited prostate carcinogenesis in TRAMP mice, reduced the number of cells expressing the SV40 large tumor antigen and proliferating cell
nuclear antigen, and increased the number of terminal dUTP nick-end labeling-positive cells in the
dorsolateral lobes of the prostate. Additionally, DIM feeding reduced the expression of cyclin A, cyclin-
dependent kinase (CDK)2, CDK4, and Bcl-xL, and increased p27 and Bax expression. To assess the
mechanisms by which DIM induces apoptosis, LNCaP and DU145 human prostate cancer cells were
cultured with various concentrations of DIM. DIM induced a substantial reduction in the numbers of
viable cells and induced apoptosis in LNCaP and DU145 cells. DIM increased the cleavage of caspase-9, -7,
-3, and poly (ADP-ribose) polymerase (PARP). DIM increased mitochondrial membrane permeability
and the translocation of cytochrome c and Smac/Diablo from the mitochondria. Additionally, DIM induced
increases in the levels of cleaved caspase-8, truncated Bid, Fas, and Fas ligand, and the caspase-8 inhibitor
Z-IETD-FMK was shown to mitigate DIM-induced apoptosis and the cleavage of caspase-3, PARP, and Bid.
These results indicate that DIM inhibits prostate carcinogenesis via induction of apoptosis and inhibition
of cell cycle progression. DIM induces apoptosis in prostate cancer cells via the mitochondria- and death
receptor-mediated pathways.


repeat and D327N polymorphism on serum sex hormone-binding globulin concentration in

SHBG is the specific plasma transport protein for sex steroid hormones in humans. Plasma SHBG
concentration follows a gender dimorphism but varies with nutritional and hormonal status in both sexes.
In addition, a genetic influence on SHBG in humans has recently been suggested by family studies. We
investigated the relationship between a point mutation (D327N) in SHBG gene exon 8 that delays human
SHBG half-life and a pentanucleotide repeat polymorphism [PNRP (TAAAA)(n)] in the SHBG gene 5'
untranslated region that influences transcription in vitro, on the one hand, and SHBG levels on the other,
in a population of 303 women referred for hirsutism. Of these patients, 154 (51%) met the criteria for
polycystic ovary syndrome (PCOS) and 124 (41%) were overweight [body mass index (BMI) > or = 25
kg/m(2)]. The two SHBG gene alleles for D327N substitution, wild-type (W) and variant (v), were identified
by restriction fragment length polymorphism in the whole population, and the GeneScan method was
used to identify PNRP alleles in 245 subjects. Six alleles of the pentanucleotide motif with six to 11 repeats
were present in our population. Plasma SHBG concentration was related to PCOS status, non-SHBG-bound
testosterone, BMI, fasting blood glucose level, fasting insulinemia, and D327N allele v. The v allele was
associated with higher SHBG levels [36.9 +/- 15.9 nmol/liter for W/v (n = 52) and 43.5 +/- 3.5 nmol/liter
for v/v (n = 2)] than was the wild-type W allele [31.1 +/- 16.1 nmol/liter (n = 249); P = 0.039]. Multivariate
analysis showed that BMI, PCOS status, and D327N polymorphism influenced plasma SHBG
concentrations, each of these parameters contributing independently of the others. Investigating the role
of each allele of the TAAAA repeat polymorphism on SHBG levels was more complex because of the
number of different genotypes (as many as 18 in our population) and the low frequency of some of them.
Moreover, a strong disequilibrium linkage was found between D327N allele v and the eight-TAAAA repeat
allele (P < 0.0001). This could mask the effect of the TAAA repeat polymorphism on SHBG concentration
in vivo. Nevertheless, SHBG levels in patients who were homozygous for six repeats (34.9 +/- 16.2
nmol/liter; n = 21) were significantly (P = 0.043) higher than in nine-repeat homozygous patients (21.5 +/-
13.0 nmol/liter; n = 8), and lay between the two for eight-repeat homozygous patients (28.5 +/- 15.8
nmol/liter; n = 44). Delineating the precise role of this PNRP polymorphism will need further investigation.
in a large healthy population. In summary, although BMI and PCOS status have a major influence on circulating SHBG levels in hirsute women, the present results support the notion that polymorphism(s) within the coding sequence and, potentially, in the regulatory sequence of the SHBG gene are associated with circulating SHBG levels and may represent part of the genetic background of sex steroid hormone activity in humans.


The effects of intraperitoneally (IP) or intracerebroventricularly (ICV) administered neurosteroids [allopregnanolone (AP); 5beta-tetrahydrodeoxy corticosterone (5beta-THDOC); dehydroepiandrosterone sulfate (DHEAS); pregnenolone sulfate (PS)] and their precursors [progesterone (PROG), pregnanediol (PREG)] on N-methyl-D-aspartic acid (NMDA)-, picrotoxin (PTX)- and bicuculline (BIC)-induced seizures and ethanol-induced sleep were studied in mice. It was found that IP injections of (+)MK-801 most potently antagonized NMDA-, PTX- and BIC-induced seizures, as compared to diazepam (DZP), PROG and PREG. Both precursors of neurosteroids appeared only marginally active in the applied models of convulsions. ICV injections of AP selectively blocked PTX- and BIC-induced seizures, whereas 5beta-THDOC and (+)MK-801 also antagonized NMDA-induced convulsions. ICV administered DHEAS induced seizures in a dose-dependent way. ICV injections of AP and midazolam shortened the latency and prolonged the duration of sleep induced by IP injections of ethanol (5.0 g/kg). On the contrary, DHEAS and PS significantly reduced the hypnotic-like effect of ethanol. The obtained results suggest that neurosteroids may modulate in an agonistic (AP, 5beta-THDOC), or antagonistic way (PS, DHEAS), the GABA(A) receptor complex functions. Some of them (5beta-THDOC) also interact with NMDA receptors. AP appeared to be the most selectively acting compound, with its profile of action fully comparable to that of midazolam. AP also enhanced the hypnotic effect of ethanol, pointing out to the propensity to interact with centrally depressant agents. These findings, together with the possibility of conversion of some neurosteroids in the brain to other steroid hormones (testosterone, estradiol and aldosterone), indicate the limitations of their use for the treatment of neurological and psychiatric disorders.


Purification by ultrafiltration of the melatonin-free pineal extract proved to have an antigonadotropic activity enabled the authors to obtain two fractions: one with a molecular weight above 10,000 and a second one with a molecular weight under 10,000 daltons. A biologic trial made by applying the mouse uterus weight test showed that both fractions inhibited the stimulating effect of exogenous HCG on the uterus. Ventral prostate weight test applied to rats showed that only the fraction above 10,000 daltons lowered the weight of this organ under basal conditions and that it inhibited the stimulating effect of exogenous testosterone on the prostate. The fraction with a molecular weight above 10,000 daltons contains 76% Lowry proteins from the total pineal extract and 4% aminic nitrogen and in the polyacrylamide gel electrophoresis it appears as two protein bands. The fraction with a molecular weight under 10,000 daltons contains only 20% proteins and 26% aminic nitrogen from the total pineal extract. Paper chromatography of the amino acids has shown that the lower molecular weight fraction does not contain proline, tyrosine, arginine, lysine and histidine and that in both fractions prevail the aspartic and

D-Aspartic acid (d-Asp), an endogenous amino acid present in vertebrates and invertebrates, plays an important role in the neuroendocrine system, as well as in the development of the nervous system. During the embryonic stage of birds and the early postnatal life of mammals, a transient high concentration of d-Asp takes place in the brain and in the retina. d-Asp also acts as a neurotransmitter/neuromodulator. Indeed, this amino acid has been detected in synaptosomes and in synaptic vesicles, where it is released after chemical (K⁺ ion, ionomycin) or electric stimuli. Furthermore, d-Asp increases cAMP in neuronal cells and is transported from the synaptic clefts to presynaptic nerve cells through a specific transporter. In the endocrine system, instead, d-Asp is involved in the regulation of hormone synthesis and release. For example, in the rat hypothalamus, it enhances gonadotropin-releasing hormone (GnRH) release and induces oxytocin and vasopressin mRNA synthesis. In the pituitary gland, it stimulates the secretion of the following hormones: prolactin (PRL), luteinizing hormone (LH), and growth hormone (GH). In the testes, it is present in Leydig cells and is involved in testosterone and progesterone release. Thus, a hypothalamus-pituitary-gonads pathway, in which d-Asp is involved, has been formulated. In conclusion, the present work is a summary of previous and current research done on the role of d-Asp in the nervous and endocrine systems of invertebrates and vertebrates, including mammals.


D-Aspartic acid (D-Asp) is an endogenous amino acid which occurs in many marine and terrestrial animals. In fetal and young rats, this amino acid occurs prevalently in nervous tissue, whereas at sexual maturity it occurs in endocrine glands and above all in pituitary and testes. Here, we have studied if a relationship exists between the presence of D-Asp and the hormonal activity. The following results were obtained: 1) Both D-Asp and testosterone are synthesized in rat testes in two periods of the animal's life: before birth, about the 17th day after fertilization and, after birth, at sexual maturity. 2) Immunocytochemical studies have demonstrated that this enantiomer is localized in Leydig and Sertoli cells. 3) In vivo experiments, consisting of i.p. injection of D-Asp to adult male rats, demonstrated that this amino acid accumulates in pituitary and testis (after 5 h, the accumulation was of 12 and 4-fold over basal values, respectively); simultaneously, luteinizing hormone, testosterone and progesterone significantly increased in the blood (1.6-fold, $p < 0.05$; 3.0-fold, $p < 0.01$ and 2.9-fold, $p < 0.01$, respectively). 4) Finally, in vitro experiments, consisting of the incubation of D-Asp with isolated testes also demonstrated that this amino acid induces the synthesis of testosterone. These results suggest that free D-Asp is involved in the steroidogenesis.

The D-isomer of aspartic acid (D-Asp) has been found in rat testes. In the present study, samples of testicular venous blood plasma, rete testis fluid, interstitial extracellular fluid, luminal fluid from the seminiferous tubules, testicular parenchymal cells, epididymal spermatozoa and peripheral blood plasma were collected and analyzed for D-Asp by two methods, an enzymatic and a chromatographic HPLC method. The two methods gave very similar results for all samples. The highest concentrations of D-Asp (about 120 nmol/ml) were found in testicular venous blood plasma, with slightly lower concentrations in rete testis fluid (95 nmol/ml) and epididymal spermatozoa (80 nmol/g wet weight). Lower levels were found in testicular parenchymal cells (which would comprise mostly spermatids and spermatocytes), luminal fluid from the seminiferous tubules and interstitial extracellular fluid (26, 23 and 11 nmol/ml respectively). However, these values were all higher than those for peripheral blood plasma (6 nmol/ml). It would appear that D-Asp is being secreted by the testis mostly into the venous blood, passing thence into the rete testis fluid and being incorporated into the spermatozoa at the time or after they leave the testis. The distribution of D-Asp is thus quite different from that of testosterone, and its role and the reason for its high concentration in the male reproductive tract remain to be elucidated.


Probes for the occurrence of endogenous D-aspartic acid (D-Asp) and N-methyl-D-aspartic acid (NMDA) in the neural complex and gonads of a protochordate, the ascidian Ciona intestinalis, have confirmed the presence of these two excitatory amino acids and their involvement in hormonal activity. A hormonal pathway similar to that which occurs in vertebrates has been discovered. In the cerebral ganglion D-Asp is synthesized from L-Asp by an aspartate racemase. Then, D-Asp is transferred through the blood stream into the neural gland where it gives rise to NMDA by means of an NMDA synthase. NMDA, in turn, passes from the neuronal gland into the gonads where it induces the synthesis and release of a gonadotropin-releasing hormone (GnRH). The GnRH in turn modulates the release and synthesis of testosterone and progesterone in the gonads, which are implicated in reproduction.


GABA, glutamate and aspartate are the predominant amino acid neurotransmitters in the mammalian brain. We have previously reported a developmental sex difference in messenger RNA levels of glutamate decarboxylase, the rate-limiting enzyme in GABA synthesis [Davis A. M. et al. (1996) Horm. Behav. 30, 538-552]. Males were found to have significantly higher levels of messenger RNA in many steroid-concentrating regions of the hypothalamus and limbic system on day 1 of life. Therefore, in this study, we have examined levels of amino acid neurotransmitters during early postnatal development in many of the same or related brain areas. We found that levels of all three transmitters change as animals age. While both GABA and aspartate concentrations increase, glutamate levels decrease. In addition, there are sex differences in the changes of these transmitters.
differences in neurotransmitter levels in several areas examined, including the ventromedial and arcuate nuclei of the hypothalamus, and the CA1 region of the hippocampus. Sex differences for GABA occur only on postnatal days 1 and 5. However, sex differences in aspartate occur later in development (postnatal day 20). The CA1 region of males has a significantly greater concentration of GABA, glutamate and aspartate than females on postnatal day 1. In addition, treatment of females with testosterone propionate on the day of birth results in increased GABA levels, suggesting that these sex differences may be the result of hormone exposure during development. We hypothesize that these hormonally mediated sex differences in amino acid transmitters early in development contribute to the establishment of sexually dimorphic neuronal architecture in the adult.


In the present study we report the occurrence of D-aspartic acid (D-Asp) in the ovary of the green frog Rana esculenta and its putative involvement in testosterone production by the gonad. In the ovary, D-Asp concentrations undergo significant variations during the main phases of the sexual cycle. In spawning females (March), its concentration was low (2.5 +/- 1.1 nmol/g ovary) and during the post-reproductive period (June) it increased and reached its peak level (58.0 +/- 10.1 nmol/g) in October. In that month, vitellogenesis occurs in a new set of ovarian follicles and continues until the next spring. The concentrations of D-Asp in the ovary and of testosterone in the ovary and in the plasma were inversely correlated during the reproductive cycle: when endogenous D-Asp was low (March), testosterone was high (36.9 +/- 4.8 ng/g ovary; 23.1 +/- 2.76 ng/ml plasma) and, in contrast, when the D-Asp concentration was high (October), the testosterone concentration was low (0.86 +/- 0.21 ng/g ovary and 5.0 +/- 1.3 ng/ml plasma). In vivo experiments, consisting of injection of D-Asp (2.0 mumol/g body weight) into the dorsal lymphatic sac of adult female frogs, demonstrated that this amino acid accumulates significantly in the ovary. After 3 h, moreover, it caused a decrease in testosterone level in the plasma of about 80%. This inhibition was reversible: within 18 h after the amino acid injection, as the D-Asp concentration in the ovary decreased, the testosterone titre was restored in both ovary and plasma. In vitro experiments, conducted in isolated ovarian follicles, confirmed this phenomenon and identified these gonadal components as the putative D-Asp targets. Other amino acids (L-Asp, D-Glu, L-Glu, D-Ala and L-Ala) used instead of D-Asp were ineffective. These findings indicate that D-Asp is involved in the control of androgen secretion by the ovary in this amphibian species, revealing a more complex system for control of this androgen synthesis than was previously believed to exist.


D-Aspartic acid (D-Asp) and nitric oxide (NO) play an important role in tuning testosterone production in the gonads of male vertebrates. In particular, D-Asp promotes either the synthesis or the release of testosterone, whereas NO inhibits it. In this study, we have investigated for the first time in birds the putative effects of D-Asp and NO on testicular testosterone production in relation to two phases of the reproductive cycle of the adult captive wild-strain mallard (Anas platyrhynchos) drake. It is a typical
seasonal breeder and its cycle consists of a short reproductive period (RP) in the spring (April-May) and a non reproductive period (NRP) in the summer (July), a time when the gonads are quiescent. The presence and the localization of D-Asp and NO in the testis and the trends of D-Asp, NO and testosterone levels were assessed during the main phases of the bird's reproductive cycle. Furthermore, in vitro experiments revealed the direct effect of exogenously administered D-Asp and NO on testosterone steroidogenesis.

METHODS: By using immunohistochemical (IHC) techniques, we studied the presence and the distributional pattern of D-Asp and NO in the testes of RP and NRP drakes. D-Asp levels were evaluated by an enzymatic method, whereas NO content, via nitrite, was assessed using biochemical measurements. Finally, immunoenzymatic techniques determined testicular testosterone levels. RESULTS: IHC analyses revealed the presence of D-Asp and NO in Leydig cells. The distributional pattern of both molecules was in some way correlated to the steroidogenic pathway, which is involved in autocrine testosterone production. Indeed, whereas NO was present only during the NRP, D-Asp was almost exclusively present during the RP. Consistently, the high testosterone testicular content occurring during RP was coupled to a high D-Asp level and a low NO content in the gonad. By contrast, in sexually inactive drakes (NRP), the low testosterone content in the gonad was coupled to a low D-Asp content and to a relatively high NO level. Consequently, to determine the exogenous effects of the two amino acids on testosterone synthesis, we carried out in vitro experiments using testis sections deriving from both the RP and NRP. When testis slices were incubated for 60 or 120 min with D-Asp, testosterone was enhanced, whereas in the presence of L-Arg, a precursor of NO, it was inhibited. CONCLUSION: Our results provide new insights into the involvement of D-Asp and NO in testicular testosterone production in the adult captive wild-strain mallard drake. The localization of these two molecules in the Leydig cells in different periods of the reproductive cycle demonstrates that they play a potential role in regulating local testosterone production.

controls, serum LH was significantly elevated in hamsters pretreated with NAL, but NMDA alone did not elevate LH. Surprisingly, LH concentrations in hamsters pretreated with NAL and then injected with NMDA were significantly lower than in hamsters receiving NAL only. Treatment with a submaximal dose of NAL (0.1 mg/kg) did not increase serum LH, nor did it reveal a stimulatory effect of subsequent NMDA treatment. The results demonstrate that the decreased sensitivity to glutamatergic agonists in the sexually active state is not a reflection of masking by inhibitory EOP mechanisms.


Estrogen synthesized in situ plays a more important role in breast cancer cell proliferation than does circulating estrogen. Aromatase is the enzyme that converts androgen to estrogen and is expressed at a higher level in breast cancer tissue than in surrounding noncancer tissue. A promising route of chemoprevention against breast cancer may be through the suppression of in situ estrogen formation using aromatase inhibitors. A diet high in fruits and vegetables may reduce the incidence of breast cancer, because they contain phytochemicals that can act as aromatase inhibitors. In our previous studies, we found that grapes and wine contain potent phytochemicals that can inhibit aromatase. We show that red wine was more effective than white wine in suppressing aromatase activity. Interestingly, our results from white wine studies suggest a weak inductive effect of alcohol on aromatase activity. On the other hand, the potent effect of anti-aromatase chemicals in red wine overcomes the weak inductive effect of alcohol in wine. Several purification procedures were performed on whole red wine to separate active aromatase inhibitors from non-active compounds. These techniques included liquid-liquid extraction, silica gel chromatography, various solid phase extraction (SPE) columns, and high performance liquid chromatography. An active Pinot Noir red wine SPE C18 column fraction (20% acetonitrile-water) was more effective than complete Pinot Noir wine in suppressing aromatase assay. This red wine extract was further analyzed in a transgenic mouse model in which aromatase was over-expressed in mammary tissue. Our gavaged red wine extract completely abrogated aromatase-induced hyperplasia and other neoplastic changes in mammary tissue. These results suggest that red wine or red wine extract may be a chemopreventive diet supplement for postmenopausal women who have a high risk of breast cancer. Further research is underway to purify and characterize the active compounds in red wine that are responsible for the inhibition of aromatase.


Eurycoma longifolia (Tongkat ali; TA) is a Malaysian shrub used to treat various illnesses including male infertility. Considering that TA is used to improve male fertility and no report regarding its safety has been published, this study investigated the effects of TA extract on various sperm functions. Semen samples of 27 patients and 13 donors were divided into two groups, washed and swim-up spermatozoa, and incubated with different concentrations of TA (1, 10, 20, 100, 2000 mug ml(-1)) for 1 h at 37 degrees C. A sample without addition of TA served as control. For washed spermatozoa, significant dose-dependent trends were found for vitality, total motility, acrosome reaction and reactive oxygen species-positive spermatozoa. However, these trends were only significant if the highest concentrations were included in the calculation. Contrary, the increase in the percentage of acrosome-reacted spermatozoa with
increasing TA concentrations is very significant \((P < 0.0001)\), and a significant difference \((P = 0.0069)\) to the control could even be recorded at 20 \(\mu g\) TA per ml. For swim-up spermatozoa, no trend could be observed. Results indicate that the TA extract has no deleterious effects on sperm functions at therapeutically used concentrations \(<2.5 \text{ } \mu g = \text{ ml}^{-1}\) however at very high concentrations of TA may have harmful effects in vitro blockquote>


1. Male hypogonadism is a major problem that starts to affect middle-aged men and has adversely effects on human sexual life. The aim of the present study was to investigate the effect of strontium fructose 1,6-diphosphate (FDP-Sr) on male hypogonadism in rats. 2. The pharmacological model of testis dysfunction was created by administration of adenine (200 mg/kg per day, i.g.) for 30 days. Three doses of FDP-Srs (200, 100 and 50 mg/kg per day, i.g.) were administered in parallel with adenine. Finally, mating behaviour index (the mounting latency and the number of mounting events), the total number of spermatozoa and sperm motility, related enzyme function and gene regulation and the mRNA levels of steroidogenic acute regulatory protein (StAR), cytochrome P450 side-chain cleavage enzyme (P450sc), 3beta-hydroxysteroid dehydrogenase (3beta-HSD), prepro-endothelin (ET)-1, endothelin-converting enzyme (ECE) and endothelin receptor A (ET(A)) were analysed. 3. The results showed that adenine significantly prolonged the mounting latency and decreased the number of mounting events, markedly reduced the total number of spermatozoa, slowed sperm motility and decreased testicular enzyme activity in the testes. At the mRNA level, adenine significantly downregulated serum testosterone, StAR, P450sc and 3beta-HSD. In parallel, adenine also targeted the ET-1 system, significantly downregulating mRNA levels of prepro-ET-1, ECE and ET(A). Administration of FDP-Sr dose-dependently reversed these effects. 4. In conclusion, adenine-induced testis dysfunction appears to be manifested as loss of sexual function in association with decreased spermatogenesis and reduced mRNA levels of steroidogenesis and the testicular ET-1 system. These abnormalities were significantly restored by FDP-Sr in a dose-dependent manner. These data indicate the possibility of using FDP-Sr to treat male hypogonadism.


Mitochondrial aspartate transamination was investigated as a major source of oxalacetate for citrate synthesis in rat ventral prostate. Citrate accumulation was measured in isolated mitochondria incubated with acetyl coenzyme A and various combinations of amino acids. Aspartate plus alpha ketoglutarate in the presence of acetyl coenzyme A resulted in significant citrate accumulation. Neither aspartate nor alpha ketoglutarate alone resulted in any significant citrate accumulation. Aspartate and alpha ketoglutarate use was comparable to glutamate and citrate production. The results indicated the presence of a mitochondrial aspartate aminotransferase. Castration (3 days) caused a significant decrease in citrate production from aspartate plus alpha ketoglutarate as well as a decrease in mitochondrial AAT activity in prostate although no effect on kidney activity occurred. A single injection of 1 mg. testosterone propionate to castrate rats significantly increased prostate mitochondrial AAT activity within 24 hours while MDH activity was unaltered. A double reciprocal plot indicated that testosterone might regulate the level of mitochondrial AAT in prostate. Ventral prostate also contain a uniquely high level of endogenous
aspartate. These studies indicate that aspartate might be the major 4-carbon source of oxalacetate for citrate synthesis. Also testosterone possibly regulated prostate citrate production by its effect on the level of mitochondrial AAT activity.


Citrate oxidation by rat ventral prostate was reduced by castration and increased by testosterone administration. Similarly, the mitochondrial aconitase activity was decreased by castration; whereas cytosol aconitase was unaffected. The rate of citrate oxidation is extremely low in prostate. Castration also decreased mitochondrial aspartate aminotransferase activity while having no effect on the cytosol isoenzyme. Testosterone markedly stimulated the net production of citrate from aspartate plus glutamate by prostate mitochondria. These studies support the proposal that aspartate is a major source of oxalacetate for citrate production, and that a "glutamate-aspartate-citrate" pathway may be functional in prostate mitochondria. In addition, testosterone can regulate citrate production by a specific effect on mitochondrial aspartate aminotransferase activity. Testosterone also regulates the flux of citrate through the Krebs cycle, but this represents only a small proportion of the citrate accumulated. These conditions would be consistent with the function of prostate epithelium in accumulating and secreting citrate.


BACKGROUND: Prostate epithelial cells accumulate a high level of aspartate that is utilized as a substrate for their unique function of production and secretion of enormously high levels of citrate. In most mammalian cells aspartate is synthesized; and, therefore is a non-essential amino acid. In contrast, in citrate-producing prostate cells, aspartate is an essential amino acid that must be derived from circulation. The prostate intracellular/extracellular conditions present a 40:1 concentration gradient. Therefore, these cells must possess a plasma membrane-associated aspartate uptake transport process to achieve their functional activity. In earlier kinetic studies we identified the existence of a unique Na+-dependent high-affinity L-aspartate transport process in rat prostate secretory epithelial cells. The present report is concerned with the identification of this putative L-aspartate transporter in rat and human prostate cells. RESULTS: The studies show for the first time that EAAC1 is expressed in normal rat prostate epithelial cells, in normal and hyperplastic human prostate glands, and in human malignant prostate cell lines. EAAC1 expression and high-affinity L-aspartate transport are correspondingly down-regulated by EAAC1 siRNA knock down. Exposure of prostate cells to physiological levels of prolactin or testosterone results in an up-regulation of EAAC1 expression and a corresponding increase in the high-affinity transport of L-aspartate into the cells. CONCLUSION: This study shows that EAAC1 functions as the high-affinity L-aspartate transporter that is responsible for the uptake and accumulation of aspartate in prostate cells. In other cells (predominantly excitable tissue cells), EAAC1 has been reported to function as a glutamate transporter rather than as an aspartate transporter. The regulation of EAAC1 expression and L-aspartate transport by testosterone and prolactin is consistent with their regulation of citrate production in prostate cells. The identification of EAAC1 as the high-affinity L-aspartate transporter now
permits studies to elucidate the mechanism of hormonal regulation of EAAC1 gene expression, and to investigate the mechanism by which the cellular environment effects the functioning of EAAC1 as an aspartate transporter or as a glutamate transporter.


D-Aspartate (D-Asp) is an endogenous amino acid present in nervous and endocrine tissues in mammals. A high concentration of D-Asp is observed in embryos, which disappears in nervous tissues after delivery, but increases temporarily in endocrine glands, particularly in the pituitary, pineal and adrenal glands at the specific stages. In the pineal gland, D-Asp that is apparently derived from other tissues suppresses melatonin secretion from parenchymal cells. Additionally, D-Asp levels increase in the testis just before birth and during maturation. The amino acid is presumed to be synthesized by the pituitary gland and testis. In the testis, D-Asp produced inside the seminiferous tubules acts on Leydig cells following release to enhance testosterone synthesis by activating the expression of Steroidogenic Acute Regulatory protein. Mammalian cells appear to contain all the molecular components required to regulate D-Asp homeostasis, as they can synthesize, release, take up, and degrade the amino acid. These findings collectively indicate that D-Asp is a novel type of messenger in the mammalian body.


This study was designed to verify whether fasting influences vascular endothelial growth factor (VEGF) production and VEGF, VEGF receptor-2 (VEGFR-2) as well as endothelin (ET) system members (endothelin converting enzyme-1, ECE-1; ET-1; endothelin receptor type A, ET-A) mRNA expression in pig corpora lutea; furthermore, we wanted to assess whether fasting affects steroidogenesis in luteal cells. Eight prepubertal gilts were induced to ovulate and were randomly assigned to two groups: (A) n = 4, normally fed; and (B) n = 4, fasted for 72 h starting 3 days after ovulation. At the end of fasting, ovaries were removed from all the animals and corpora lutea (CLs) were collected. VEGF and steroid levels in luteal tissue were determined by ELISA and RIA, respectively; VEGF, VEGFR-2, ET-1, ET-A and ECE-1 mRNAs expression was measured by real-time PCR. VEGF protein levels were similar in the two groups, while all steroid (progesterone, testosterone, estradiol 17beta) concentrations were significantly (P < 0.001) higher in CLs collected from fasted animals compared with those from normally fed gilts. VEGF, VEGFR-2, ET-1 and ECE-1 (but not ET-A) mRNA expression was significantly lower (P < 0.05) in fasted versus normally fed animals. The overall conclusion is that all the parameters studied are affected by feed restriction, but the mechanisms activated at luteal level are possibly not fully adequate to compensate for nutrient shortage.

Epidemiologic evidence suggests that high dietary intake of Brassica vegetables, such as broccoli, cabbage, and Brussels sprouts, protects against tumorigenesis in multiple organs. 3,3'-Diindolylmethane, one of the active products derived from Brassica vegetables, is a promising antitumor agent. Previous studies in our laboratory showed that 3,3'-diindolylmethane induced a G(1) cell cycle arrest in human breast cancer MCF-7 cells by a mechanism that included increased expression of p21. In the present study, the upstream events leading to p21 overexpression were further investigated. We show for the first time that 3,3'-diindolylmethane is a strong mitochondrial H(+)-ATPase inhibitor (IC(50) approximately 20 micromol/L). 3,3'-Diindolylmethane treatment induced hyperpolarization of mitochondrial inner membrane, decreased cellular ATP level, and significantly stimulated mitochondrial reactive oxygen species (ROS) production. ROS production, in turn, led to the activation of stress-activated pathways involving p38 and c-Jun NH(2)-terminal kinase. Using specific kinase inhibitors (SB203580 and SP600125), we showed the central role of p38 and c-Jun NH(2)-terminal kinase (JNK) pathways in 3,3'-diindolylmethane-induced p21 mRNA transcription. In addition, antioxidants significantly attenuated 3,3'-diindolylmethane-induced activation of p38 and JNK and induction of p21, indicating that oxidative stress is the major trigger of these events. To further support the role of ROS in 3,3'-diindolylmethane-induced p21 overexpression, we showed that 3,3'-diindolylmethane failed to induce p21 overexpression in mitochondrial respiratory chain deficient rho(0) MCF-7 cells, in which 3,3'-diindolylmethane did not stimulate ROS production. Thus, we have established the critical role of enhanced mitochondrial ROS release in 3,3'-diindolylmethane-induced p21 up-regulation in human breast cancer cells.


We have previously reported that a synthetic peptide amide corresponding to residues 34-37 (TRDL, threonine, arginine-aspartic acid-leucine) of the beta-subunit of human FSH induced prolonged vaginal estrus in normally cycling female mice (see Ref. 15). These results represented the first demonstration of an in vivo effect of a gonadotropin-related synthetic peptide on reproductive processes. We have extended these studies to examine possible effects of TRDL on the onset of puberty in female mice. In two replicated experiments, vehicle-injected control mice attained first vaginal estrus by day 39. An ip injection of 200 ug TRDL/g BW to 28-day-old prepubertal female mice, however, accelerated the onset of first vaginal estrus by 7 days in 11 of 12 (11/12) (Exp 1) and 7/9 (Exp 2) mice. Serum estradiol levels were significantly (P = 0.017) elevated in TRDL-treated mice, whereas progesterone was unchanged. Uteri of TRDL-treated mice were significantly (P = 0.003) heavier than uteri of vehicle-injected control animals of the same age and body weight. Intraluminal fluid accumulation (ballooning) at proestrus was absent in 20/21 TRDL-treated females, as were oviductal ova and ovarian corpora lutea. These phenomena are characteristic of the first estrous cycles of female mice isolated from males. To obtain further evidence for
in vivo effects of TRDL, we assessed the ability of TRDL to accelerate the onset of puberty in male mice. When given as five consecutive daily ip injections of 200 ug/g BW to 28-day-old prepubertal male mice, TRDL significantly increased testis weight, when compared with vehicle-injected control mice of the same age and BW (171.3 +/- 3.8 mg vs. 151.6 +/- 4.3 mg, P = 0.001) and induced a 6.5-fold increase in serum testosterone levels. These studies confirm the previously reported in vivo activity of a synthetic peptide corresponding to human FSH-beta subunit 34-37 (TRDL) in adult female mice and extend its effects to the acceleration of the onset of puberty in immature male and female mice.


In the striated ducts of the sublingual glands of normal adult male, but not female, Swiss-Webster mice a few scattered cells have apical secretion granules. These sublingual duct cells resemble the granular convoluted tubule (GCT) cells of the submandibular glands of adult female mice, in that they are smaller than submandibular GCT cells of adult males, and contain fewer apical granules, and prominent basal striations. These cells stain immunocytochemically for epidermal growth factor (EGF), renin, and protease A. Such granular striated duct cells could be induced in the sublingual glands of adult female mice by treatment with either testosterone propionate or thyroxine; the two hormones given simultaneously acted synergistically in this induction.


As both gonadotropins, LH and FSH, are required for normal spermatogenesis, patients with pituitary insufficiency need hCG plus human menopausal gonadotropin therapy to induce spermatogenesis and establish fertility. In a patient hypophysectomized because of a pituitary tumor, who, despite undetectable serum gonadotropin levels, had normal testis volume and semen parameters and fathered three children under testosterone substitution alone, we hypothesized an activating mutation of the FSH receptor. Exon 10 of the FSH receptor gene was amplified from genomic DNA by PCR, screened by single stranded conformation polymorphism gel electrophoresis, and sequenced. We identified a heterozygous A-->G base change at nucleotide position 1700, leading to an Asp-->Gly transition in codon 567 in the third intracytoplasmatic loop. COS-7 cells transiently transfected with the mutated receptor displayed a 1.5-fold increase in basal cAMP production compared to wild-type receptor, indicating that this mutation leads to ligand-independent constitutive activation of the FSH receptor. We conclude that this activating mutation of the FSH receptor, the first ever described, autonomously sustains spermatogenesis in the absence of gonadotropins.

Guengerich, F. P., G. P. Miller, et al. (2002). "Diversity in the oxidation of substrates by cytochrome P450 2D6: lack of an obligatory role of aspartate 301-substrate electrostatic
Cytochrome P450 (P450) 2D6 was first identified as the polymorphic human debrisoquine hydroxylase and subsequently shown to catalyze the oxidation of a variety of drugs containing a basic nitrogen. Residue Asp301 has been characterized as being involved in electrostatic interactions with substrates on the basis of homology modeling and site-directed mutagenesis experiments [Ellis, S. W., Hayhurst, G. P., Smith, G., Lightfoot, T., Wong, M. M. S., Simula, A. P., Ackland, M. J., Sternberg, M. J. E., Lennard, M. S., Tucker, G. T., and Wolf, C. R. (1995) J. Biol. Chem. 270, 29055-29058]. However, pharmacophore models based on the role of Asp301 in substrate binding are compromised by reports of catalytic activity toward substrates devoid of a basic nitrogen, which have generally been ignored. We characterized a high-affinity ligand for P450 2D6, also devoid of a basic nitrogen atom, spirosulfonamide [4-[3-(4-fluorophenyl)-2-oxo-1-oxaspiro[4.4]non-3-en-4-yl]benzenesulfonamide], with K(s) 1.6 microM. Spirosulfonamide is a substrate for P450 2D6 (k(cat) 6.5 min(-1)) for the formation of a syn spiromethylene carbinol, K(m) 7 microM. Mutation of Asp301 to neutral residues (Asn, Ser, Gly) did not substantially affect the binding of spirosulfonamide (K(s) 2.5-3.5 microM). However, the hydroxylation of spirosulfonamide was attenuated in these mutants to the same extent (90%) as for the classic nitrogenous substrate bufuralol, and the effect of the D301N substitution was manifested on k(cat) but not K(m). Analogues of spirosulfonamide were also evaluated as ligands and substrates. Analogues in which the sulfonamide moiety was modified to an amide, thioamide, methyl sulfone, or hydrogen were ligands with K(s) values of 1.7-32 microM. All were substrates, and the methyl sulfone analogue was oxidized to the syn spiromethylene carbinol analogue of the major spirosulfonamide product. The D301N mutation produced varying changes in the oxidation patterns of the spirosulfonamide analogues. The peptidomeric ritonavir and the steroids progesterone and testosterone had been reported to be substrates for P450 2D6, but the affinities (K(s)) were unknown; these were estimated to be 1.2, 1.5, and 15 microM, respectively (cf. 6 microM for the classic substrate bufuralol). The results are consistent with a role of Asp301 other than electrostatic interaction with a positively charged ligand. H-Bonding or electrostatic interactions probably enhance binding of some substrates, but our results show that it is not required for all substrates and explain why predictive models fail to recognize the proclivity for many substrates, especially those containing no basic nitrogen.

Thus, the HFE H63D mutation seems to be an important risk factor for impaired sperm motility and is clinically associated with male infertility.


FSH mediates its testicular actions via a specific Sertoli cell G protein-coupled receptor. We created a novel transgenic model to investigate a mutant human FSH receptor (FSHR(+)) containing a single amino acid substitution (Asp567Gly) equivalent to activating mutations in related glycoprotein hormone receptors. To examine the ligand-independent gonadal actions of FSHR(+), the rat androgen-binding protein gene promoter was used to direct FSHR(+) transgene expression to Sertoli cells of gonadotropin-deficient hypogonadal (hpg) mice. Both normal and hpg mouse testes expressed FSHR(+) mRNA. Testis weights of transgenic FSHR(+) hpg mice were increased approximately 2-fold relative to hpg controls (P < 0.02) and contained mature Sertoli cells and postmeiotic germ cells absent in controls, revealing FSHR(+) -initiated autonomous FSH-like testicular activity. Isolated transgenic Sertoli cells had significantly higher basal (approximately 2-fold) and FSH-stimulated (approximately 50%) cAMP levels compared with controls, demonstrating constitutive signaling and cell-surface expression of FSHR(+), respectively. Transgenic FSHR(+) also elevated testosterone production in hpg testes, in the absence of circulating LH (or FSH), and it was not expressed functionally on steroidogenic cells, suggesting a paracrine effect mediated by Sertoli cells. The FSHR(+) response was additive with a maximal testosterone dose on hpg testicular development, demonstrating FSHR(+) activity independent of androgen-specific actions. The FSHR(+) response was male specific as ovarian expression of FSHR(+) had no effect on hpg ovary size. These findings reveal transgenic FSHR(+) stimulated a constitutive FSH-like Sertoli cell response in gonadotropin-deficient testes, and pathways that induced LH-independent testicular steroidogenesis. This novel transgenic paradigm provides a unique approach to investigate the in vivo actions of mutated activating gonadotropin receptors.


In order to extend our analysis of the reactions that occur during the active site directed photoactivation of delta 5-3-ketosteroid isomerase sensitized by unsaturated steroid ketone photoaffinity reagents, the site of covalent attachment has been identified. A solid-phase photoaffinity reagent, delta 6-testosterone-agarose, has been employed for this purpose; this type of reagent, in contrast to solution-phase reagents, facilitated the recovery of a peptide fragment of the isomerase bearing the residue at which covalent attachment had occurred. Amino acid analysis and sequence determination of the peptide provided evidence that the site of attachment was aspartate-38. This result, in combination with the low-resolution crystallographic structure of the enzyme [Westbrook, E. M., Piro, O. E., & Sigler, P. B. (1984) J. Biol. Chem. 259, 9096-9103], suggests that aspartate-38 is located in the vicinity of the bottom of the steroid-binding pit. The potential usefulness of solid-phase photoaffinity reagents in the identification of sites of covalent attachment on target proteins such as hormone receptors is discussed.
In the nonamyloidogenic processing pathway the Alzheimer's amyloid precursor protein (APP) is proteolytically cleaved by alpha-secretase. As this cleavage occurs at the Lys16-Leu17 bond within the amyloid beta domain, it prevents deposition of intact amyloidogenic peptide. In addition, the large ectodomain (sAPP(\alpha)) released by the action of alpha-secretase has several neuroprotective properties. Studies with a range of hydroxamic acid-based compounds, such as batimastat, indicate that alpha-secretase is a zinc metalloproteinase, and members of the ADAM family of proteins, TACE, ADAM10 and ADAM9, all fulfill some of the criteria required of alpha-secretase. APP is constitutively cleaved by alpha-secretase in most cell lines. However, on stimulation with muscarinic agonists or activators of protein kinase C, such as phorbol esters, the alpha-secretase cleavage of APP is up-regulated. The constitutive alpha-secretase activity is primarily at the cell surface, while the regulated activity is predominantly located within the Golgi. The beneficial action of cholinesterase inhibitors may in part be due to activation of muscarinic receptors, resulting in an up-regulation of alpha-secretase. Other agents can also increase the nonamyloidogenic cleavage of APP including estrogen, testosterone, various neurotransmitters and growth factors. As the alpha-secretase cleavage of APP both precludes the deposition of the amyloid beta peptide and releases the neuroprotective sAPP(\alpha), pharmacological up-regulation of alpha-secretase may provide alternative therapeutic approaches for Alzheimer's disease.


OBJECTIVE: To investigate the effects of long-term low dose hormone replacement therapy (HRT) on postmenopausal women in hormone level, cognition score, hippocampus volume, and magnetic resonance spectroscopy (MRS) parameters. METHODS: A total of 182 postmenopausal women aged 50-87 years were chosen at Peking Union Medical College Hospital and assigned to HRT group and control group. The volunteers of HRT group had taken low dose hormone [estradiol (E2) 0.5-1.0 mg and progesterone 0.5-2.0 mg, once a day] for 4-33 years. The concentrations of E2, progesterone, and testosterone were measured using enzyme-linked immunosorbent assay (ELISA). The gene types of apolipoprotein E (ApoE) were measured by polymerase chain reaction, and the subjects with susceptible genes (ApoE epsilon3/epsilon4) of Alzheimer's disease (AD) were screened. Their hippocampus volumes and MRS parameters were obtained through magnetic resonance imaging (MRI), and results in two groups were analyzed by statistical method. RESULTS: Compared with control group, the concentrations of E2 at each age stage in HRT group were significantly higher (P < 0.05) except the 80-89 years old subgroup; yet, there were no statistical differences in the concentrations of progesterone and testosterone between the two groups. There was no obvious difference in ApoE subtypes distribution between the two groups. The results of hippocampus MRI for the subjects with susceptible genes ApoE epsilon3/epsilon4 (HRT group 14 cases, control group 11 cases) showed that the ratio of bilateral
hippocampus volume to whole brain volume in HRT group (0.406 +/- 0.028) was significantly higher than control group (0.369 +/- 0.031, P < 0.05). The results of 1H MRS for the subjects with susceptible genes ApoE epsilon3/epsilon4 (HRT group 12 cases, control group 11 cases) showed that the N-acetylaspartate/total creatine at the area of hippocampus in HRT group (1.54 +/- 0.08) were significantly higher than control group (1.45 +/- 0.13, P < 0.05). CONCLUSIONS: For postmenopausal women, long-term low dose HRT can maintain the physiological concentration of E2 in plasma. Furthermore, the hippocampus MRI performed on those with ApoE epsilon3/epsilon4 genes shows that long-term low dose HRT can prevent hippocampus atrophy, which is beneficial to maintain the brain function and prevent AD.


D-aspartate, an abundant D-amino acid enriched in neuroendocrine tissues, can be degraded by D-aspartate oxidase (Ddo). To elucidate the function of D-aspartate, we generated mice with targeted deletion of Ddo (Ddo(-/-)) and observe massive but selective augmentations of D-aspartate in various tissues. The pituitary intermediate lobe, normally devoid of D-aspartate from endogenous Ddo expression, manifests pronounced increases of immunoreactive D-aspartate in Ddo(-/-) mice. Ddo(-/-) mice show markedly diminished synthesis and levels of pituitary proopiomelanocortin/alpha-MSH, associated with decreased melanocortin-dependent behaviors. Therefore, Ddo is the endogenous enzyme that degrades D-aspartate, and Ddo-enriched organs, low in D-aspartate, may represent areas of high turnover where D-aspartate may be physiologically important.


We hypothesized that administration of an antisense oligodeoxynucleotide (ODN) to estrogen receptor (ER)-alpha mRNA decreases the ER protein in the neonatal rat brain, alters the sex-specific ventilatory responses to aspartic acid in rats, and counteracts the effects of testosterone proportionate (TP) in females. One-day-old rat pups were injected intraventricularly with vehicle, antisense ER ODN, or scrambled ODN control. Additional groups of females received TP or vehicle and one of the three treatments. Brain ER protein levels were decreased by 65% at 6 h and 35% at 24 h after antisense ODN. Aspartic acid decreased ventilation in all groups of weanling males and females except ER ODN-treated females and TP-vehicle-treated females. Aspartic acid decreased ventilation in all groups of adult females except those given TP and in males. Weanling ER ODN-treated rats were shorter and weighed less than controls. Only adult ER ODN-treated males exhibited these traits. Thus neonatal ER affects aspartic acid modulation of breathing and body growth in a sex-specific and developmental manner.

A radiochemical method for the determination of the amino terminus on very small amounts (0.5-5 nmol) of protein is described. The high sensitivity of the method is achieved by using undiluted 1-fluoro-2,4-dinitro-[3,5-3H]benzene ([ 3H]Dnp-F) as the labelling reagent under conditions in which a maximum amount of radioactive label is incorporated. Chemical homogeneity is achieved by reacting with excess unlabelled Dnp-F. High recovery is obtained by adding Dnp-albumin as carrier protein. A mixture of Dnp 14C-labelled amino acids is added prior to hydrolysis and identification of the amino terminus is made on the basis of the 3H/14C ratios of the separated Dnp-amino acids. The method was tested on insulin, pancreatic ribonuclease, and lysozyme which gave high 3H/14C ratios only in the expected amino-terminal amino acids. Application to multiple forms of poly(C)-avid ribonuclease gave only amino-terminal lysine. Two of four putative isozymes of 17 beta-hydroxysteroid dehydrogenase had serine as the amino terminus while the other two had aspartic acid or asparagine.


Several naturally occurring and synthetic flavones were found to inhibit the aromatization of androstenedione and testosterone to estrogens catalyzed by human placental and ovarian microsomes. These flavones include (in order of decreasing potency) 7,8-benzoflavone, chrysin, apigenin, flavone, flavanone, and quercetin; 5,6-benzoflavone was not inhibitory. 7,8-Benzoflavone and chrysin were potent competitive inhibitors and induced spectral changes in the aromatase cytochrome P-450 indicative of substrate displacement. Flavones may thus compete with steroids in their interaction with certain monooxygenases and thereby alter steroid hormone metabolism.


Aromatase, the key enzyme in estrogen biosynthesis, converts androstenedione to estrone and testosterone to estradiol. The enzyme is expressed in various tissues such as ovary, placenta, bone, brain, skin, and adipose tissue. Aromatase enzyme is encoded by a single gene CYP 19A1 and its expression is controlled by tissue-specific promoters. Aromatase mRNA is primarily transcribed from promoter I.4 in normal breast tissue and physiological levels of aromatase are found in breast adipose stromal fibroblasts. Under the conditions of breast cancer, as a result of the activation of a distinct set of aromatase promoters (I.3, II, and I.7) aromatase expression is enhanced leading to local overproduction of estrogen that promotes breast cancer. Aromatase is considered as a potential target for endocrine treatment of breast cancer but due to nonspecific reduction of aromatase activity in other tissues, aromatase inhibitors (AIs) are associated with undesirable side effects such as bone loss, and abnormal lipid metabolism. Inhibition of aromatase expression by inactivating breast tumor-specific aromatase promoters can selectively block estrogen production at the tumor site. Although several synthetic chemical compounds and nuclear receptor ligands are known to inhibit the activity of the tumor-specific aromatase promoters, further development of more specific and efficacious drugs without adverse effects is still warranted. Plants are rich in chemopreventive agents that have a great potential to be used in chemotherapy for...
hormone dependent breast cancer which could serve as a source for natural AIs. In this brief review, we summarize the studies on phytochemicals such as biochanin A, genistein, quercetin, isoliquiritigenin, resveratrol, and grape seed extracts related to their effect on the activation of breast cancer-associated aromatase promoters and discuss their aromatase inhibitory potential to be used as safer chemotherapeutic agents for specific hormone-dependent breast cancer.


BACKGROUND: 3,3-Diindolylmethane (DIM) is a major in vivo product of acid-catalyzed oligomerization of indole-3-carbinol (I3C) derived from Brassica food plants. Although DIM is known as a chemopreventive and chemotherapeutic phytochemical, the effects of DIM on inflammation in vivo are still unknown. In the present study we investigated the antiinflammatory effects of DIM on experimental colitis and colitis-associated colorectal carcinogenesis. METHODS: To determine if DIM has an antiinflammatory effect in vivo, we examined the therapeutic effects of DIM in dextran sodium sulfate (DSS)-induced experimental colitis and colitis-associated colon carcinogenesis induced by azoxymethane (AOM)/DSS in BALB/c mice. RESULTS: Treatment with DIM significantly attenuated loss of body weight, shortening of the colon, and severe clinical signs in a colitis model. This was associated with a remarkable amelioration of the disruption of the colonic architecture and a significant reduction in colonic myeloperoxidase activity and production of prostaglandin E(2), nitric oxide, and proinflammatory cytokines. Further, DIM administration dramatically decreased the number of colon tumors in AOM/DSS mice. CONCLUSIONS: These results suggest that DIM-mediated antiinflammatory action at colorectal sites may be therapeutic in the setting of inflammatory bowel disease and colitis-associated colon cancer.


Male mice were castrated at 2 mo and a pellet of testosterone propionate was implanted subcutaneously 2 wk later. Mice were killed after 11 days and tissue extracts were analyzed. All the common 20 amino acids were present in widely varying concentrations. Castration uniformly increased the concentration of all free amino acids of the seminal vesicle, except proline, cystine/2, and tryptophan; androgen restored values to normal. These changes were not entirely due to changes in quantity of seminal vesicle fluid. Concentrations of amino acids of the prostate were not significantly changed by castration or testosteronepropionate. Fifteen other ninhydrin-postive compounds were detected. Hypotaurine, taurine alpha-aminobutyric acid, and cysteic acid in seminal vesicles were greatly decreased and several other compounds were slightly decreased by castration and restored tp normal by testosterone propionate. In the prostate, hypotaurine, alpha-aminobutyric acid, cysteic/cysteinesulfinic acids, glycerophosphoethanomine, cystathionine, and phosphoethanolamine were decreased and alpha-amino-n-butyric acid was increased by castration and restored to normal by testosterone propionate. Concentrations of taurine and 5 other compounds were not affected. Epididymis and testis also contained appreciable pools of amino acids and the other compounds. Amino acids concentrations were lower in cauda than in caput epididymis; in the testis values were intermediate, with much higher concentrations of glutamic and aspartic acids. Taurine concentrations were twice as great in cauda as in caput epididymis; concentrations of hypotaurine and the other sulfur-containing compounds were similar in both parts of the epididymis. The most striking result in the testis was the high concentration of reduced glutathione.
The 17beta-hydroxysteroid dehydrogenase type 5 (17beta-HSD 5) is involved in estrogen and androgen metabolism. In our study we tested the influence of environmental hormones, such as phytoestrogens (flavonoids, coumarins, coumestans), on reductive and oxidative 17beta-HSD activity of the human 17beta-hydroxysteroid dehydrogenase type 5 (17beta-HSD 5). These dietary substances were shown to be potent inhibitors of aromatase, different 17beta-HSDs and seem to play an important role in delay of development of hormone dependent cancers. Our studies show that reductive and oxidative activity of the enzyme are inhibited by many dietary compounds, especially zearalenone, coumestrol, quercetin and biochanin A. Among the group of flavones inhibitor potency is growing with increasing number of hydroxylations. We suggest that these substances are bound to the hydrophilic cofactor-binding pocket of the enzyme. An interesting inhibition pattern is observed for 18beta-glycyrrhetinic acid, which has no influence on the oxidative but only on the reductive reaction. This indicates that this substrate binds to pH- and cofactor-depending sites at the active center of the enzyme.

Phytoestrogens contained in a vegetarian diet are supposed to have beneficial effects on the development and progression of a variety of endocrine-related cancers. We have tested the effect of a variety of dietary phytoestrogens, especially flavonoids, on the activity of human 17beta-hydroxysteroid dehydrogenase type 5 (17beta-HSD 5), a key enzyme in the metabolism of estrogens and androgens. Our studies show that reductive and oxidative activity of the enzyme are inhibited by many compounds, especially zearalenone, coumestrol, quercetin and biochanin A. Among flavones, inhibitor potency is enhanced with increased degree of hydroxylation. The most effective inhibitors seem to bind to the hydrophilic cofactor binding pocket of the enzyme.

There is evidence that certain phytoestrogens can inhibit key steroidogenic enzymes although most studies have been carried out on microsomal or purified enzyme preparations, some using cell lines. This study was designed to test the hypothesis that low doses of phytoestrogens, at concentrations that would be attained through the diet, could inhibit 3beta-hydroxysteroid dehydrogenase (HSD) and/or aromatase in primary cultures of human granulosa-luteal (GL) cells and that this effect was due to a decrease in the expression of these proteins. Based on published evidence, eight compounds were selected for investigation and these included the flavones apigenin and quercetin, the isoflavones genistein, biochanin A and daidzein, the lignans, enterodiol and enterolactone, and the mycotoxin zearalenone. Human GL cells were cultured for 48 h in the presence of these phytoestrogens at concentrations ranging from 0.01 to 100 microM and after addition of fresh media the conversion of pregnenolone to progesterone or...
androstenedione to oestradiol over a 4h period was measured. Biochanin A was the only phytoestrogen that displayed any dose-dependent inhibition of 3beta-HSD, others showing inhibition at doses >/=10 microM. Apigenin and quercetin only inhibited aromatase/17beta-HSD at high doses as did genistein, biochanin A and daidzein. The lignans had weak inhibitory effects on aromatase/17beta-HSD, whilst zearalenone showed potent inhibition at 0.1 microM. Phytoestrogens did not exert any significant effects on protein expression of 3beta-HSD or aromatase as determined by Western blots. It is concluded that steroidogenic enzymes are inhibited by phytoestrogens in primary cultures of human GL cells but these cells are less sensitive to the effects of phytoestrogens than cell-free systems. This may be due to poor lipid solubility or cellular metabolism. We have also shown for the first time that phytoestrogens do not act by inhibiting the cellular concentration of 3beta-HSD and aromatase even though exposure time would have allowed for changes in gene expression.


D-aspartic acid (D-Asp), aromatase enzyme activity and the putative D-Asp involvement on aromatase induction have been studied in the testis of mature boars. The peroxidase-antiperoxidase and the indirect immunofluorescence methods, applied to cryostat and paraffin sections, were used to evaluate D-Asp and aromatase distributions. D-Asp level was dosed by an enzymatic method performed on boar testis extracts. Biochemical aromatase activity was determined by in vitro experiments carried out on testis extracts. D-Asp immunoreactivity was found in Leydig cells, and, to a lesser extent, in germ cells. Analogously, aromatase immunoreactivity was present in Leydig cells, but absent from seminiferous tubule elements. In vitro experiments showed that the addition of D-Asp to testicular tissue acetone powder induced a significant increase of aromatase activity, as assessed by testosterone conversion to 17beta-estradiol. Enzyme Km was not affected by D-Asp (about 25 nM in control and D-Asp added tests). These findings suggest that D-Asp could be involved in the local regulation of aromatase in boar Leydig cells and intervenes in this organ's production of estrogens.


Mammalian testis contains D-aspartic acid (D-Asp), which enhances testosterone production. D-Asp, on other hand, also stimulates 17beta-estradiol synthesis in the ovary of some lower vertebrates. We studied boar testis in order to determine if D-Asp intervenes in 17beta-estradiol synthesis in the testis of those mammals which produce significant amounts of estrogens as well as testosterone. The boar testis contains D-Asp (40 +/- 3.6 nmol/g tissue) which, according to immunohistological techniques, is localized mainly in Leydig cells, and, to a lesser extent, in sustentacular (Sertoli), peritubular and some germ cells. The enzyme P450aromatase is present in Leydig cells and few germ cells. In vitro experiments showed that the addition of D-Asp to testicular tissue extracts induced a significant increase of aromatase activity, as evaluated by testosterone conversion to 17beta-estradiol. The enzyme's K(m) was not affected by D-Asp (about 25 nM in both control and D-Asp added tests). On the basis of these results we suggest that, as in the ovary, D-Asp is involved in the local control of aromatase activity of boar testis and, therefore, it intervenes in the 17beta-estradiol production. In the testis, the D-Asp targets are presumably the Leydig cells, which having also a nuclear estrogen receptor are, in turn, one of the putative targets of the 17beta-estradiol that they produce (autocrine effect).

D-Aspartic acid (D-Asp) and nitric oxide (NO) are two biologically active molecules playing important functions as neurotransmitters and neuromodulators of nerve impulse and as regulators of hormone production by endocrine organs. We studied the occurrence of D-Asp and NO as well as their effects on testosterone synthesis in the testis of boar. This model was chosen for our investigations because it contains more Leydig cells than other mammals. Indirect immunofluorescence applied to cryostat sections was used to evaluate the co-localization of D-Asp and of the enzyme nitric oxide synthase (NOS) in the same Leydig cells. D-Asp and NOS often co-existed in the same Leydig cells and were found, separately, in many other testicular cytotypes. D-Asp level was dosed by an enzymatic method performed on boar testis extracts and was 40+/-.3.6 nmol/g of fresh tissue. NO measurement was carried out using a biochemical method by NOS activity determination and expressed as quantity of nitrites produced: it was 155.25+/-21.9 nmol/mg of tissue. The effects of the two molecules on steroid hormone production were evaluated by incubating testis homogenates, respectively with or without D-Asp and/or the NO-donor L-arginine (L-Arg). After incubation, the testosterone presence was measured by immunoenzymatic assay (EIA). These in vitro experiments showed that the addition of D-Asp to incubated testicular homogenates significantly increased testosterone concentration, whereas the addition of L-Arg decreased the hormone production. Moreover, the inclusion of L-Arg to an incubation medium of testicular homogenates with added D-Asp, completely inhibited the stimulating effects of this enantiomer. Our results suggest an autocrine action of both D-Asp and NO on the steroidogenetic activity of the Leydig cell.


The prostate gland produces and secretes extraordinarily high levels of citrate. Studies with rat ventral prostate (VP) have demonstrated that aspartate can serve as a four-carbon source of oxalacetate in the synthesis of citrate. To achieve this, prostate secretory epithelial cells must contain a transport system for the active uptake of aspartate from circulation. The present studies with VP epithelial cells confirm the existence of a Na(+)-dependent high-affinity L-aspartate transporter. The transporter has an optimal pH approximately 7.5 and is temperature dependent. It appears to be an anionic amino acid transporter capable of transporting L-glutamate but not basic or neutral amino acids. The transporter is inhibited by ATPase inhibitors, thereby indicating its dependency on a Na+ gradient. The characteristics of the high-affinity L-aspartate transporter are consistent with its operation at the basilar membrane for the transport of circulating aspartate into the cell. Castration (24 hr) resulted in a significant decrease in the ability of VP epithelial cells to transport L-aspartate. The administration of testosterone to castrated rats completely restored L-aspartate transport. In addition, in vitro testosterone addition (10(-8) M for 30 min) to isolated prostate epithelial cells markedly increased L-aspartate transport. Both cycloheximide and actinomycin inhibited the testosterone effect. The studies reveal that testosterone is a regulator of this Na(+)-dependent high-affinity L-aspartate transporter. The mechanism of this testosterone effect appears to involve both RNA and protein synthesis. We now have a model system to elucidate this novel effect of testosterone.

Le, H. T., C. M. Schaldach, et al. (2003). "Plant-derived 3,3′-Diindolylmethane is a strong..."
3,3'-Diindolylmethane (DIM) is a major digestive product of indole-3-carbinol, a potential anticancer component of cruciferous vegetables. Our results indicate that DIM exhibits potent antiproliferative and antiandrogenic properties in androgen-dependent human prostate cancer cells. DIM suppresses cell proliferation of LNCaP cells and inhibits dihydrotestosterone (DHT) stimulation of DNA synthesis. These activities were not produced in androgen-independent PC-3 cells. Moreover, DIM inhibited endogenous PSA transcription and reduced intracellular and secreted PSA protein levels induced by DHT in LNCaP cells. Also, DIM inhibited, in a concentration-dependent manner, the DHT-induced expression of a prostate-specific antigen promoter-regulated reporter gene construct in transiently transfected LNCaP cells. Similar effects of DIM were observed in PC-3 cells only when these cells were co-transfected with a wild-type androgen receptor expression plasmid. Using fluorescence imaging with green fluorescent protein androgen receptor and Western blot analysis, we demonstrated that DIM inhibited androgen-induced androgen receptor (AR) translocation into the nucleus. Results of receptor binding assays indicated further that DIM is a strong competitive inhibitor of DHT binding to the AR. Results of structural modeling studies showed that DIM is remarkably similar in conformational geometry and surface charge distribution to an established synthetic AR antagonist, although the atomic compositions of the two substances are quite different. Taken together with our published reports of the estrogen agonist activities of DIM, the present results establish DIM as a unique bifunctional hormone disrupter. To our knowledge, DIM is the first example of a pure androgen receptor antagonist from plants.


The effects of glutamate on the in vitro basal steroid production of three maturational stages of rainbow trout (Oncorhynchus mykiss) ovarian follicles were investigated. Radioimmunoassays were used to measure the rates of synthesis of testosterone (T) and 17-estradiol (E2). High performance liquid chromatography (HPLC) was used to examine the steroid metabolites produced from a tritium labeled precursor, pregnenolone (P5). The glutamate agonist, N-methyl-d,l-aspartate (NMA) had a dose-dependent suppressive effect on T and E2 synthesis in mid-vitellogenic (MV) follicles, but had no significant effect on early- (EV) and late-vitellogenic (LV) follicles. L-glutamic acid (GA) had a dose-related suppressive effect on T synthesis by MV follicles, suppressing both T and E2 synthesis by LV follicles, but having no effect on EV follicles. HPLC separation of the steroid metabolites synthesized from P5 showed clear evidence of differences in rates of overall steroidogenesis of the three follicular stages, but no effect of either NMA or GA on the nature or the amount of the metabolites produced by the three developmental stages examined. The findings suggest that glutamate may act via a reduction in the production of P5, which is the principal rate-limiting step in the steroidogenic cascade, and not via modulation of steroidogenic enzyme activities.

Ledda, A., G. Belcaro, et al. (2010). "Investigation of a complex plant extract for mild to moderate erectile dysfunction in a randomized, double-blind, placebo-controlled, parallel-arm
OBJECTIVE: To assess the effects of a complex plant extract (Prelox(R), a formulation of pine bark extract and L-arginine aspartate; Horphag Research UK Ltd, London, UK) on erectile dysfunction (ED) in men, as sexual desire typically persists in ageing men, while their erectile and endothelial function gradually declines. PATIENTS AND METHODS: In this double-blind, placebo-controlled study we assessed the effects of Prelox in 124 patients (aged 30-50 years) with moderate ED over an investigational period of 6 months. The International Index Of Erectile Function (IIEF) was used to quantify changes in sexual function. RESULTS: The erectile domain of the IIEF (questions 1-5 plus 15) improved with Prelox from a baseline mean (sd) score of 15.2 (6.6) to 25.2 (2.1) after 3 months and 27.1 (2.1) after 6 months of treatment. In the placebo group there was an increase from a baseline score of 15.1 (7.0) to 19.1 (3.0) and 19.0 (3.1) after 3 and 6 months, respectively. The effects with Prelox were statistically significant compared with placebo (P < 0.05). Mean (SD) total plasma testosterone levels increased significantly from 15.9 (2.3) to 18.9 (2.6) nmol/L (P < 0.05) after 6 months with Prelox, compared to an increase from 16.9 (2.4) to 17.3 (2.3) nmol/L in the placebo group. CONCLUSION: This study shows that Prelox is effective for improving erectile function, and that this effect persists on continuous therapy for up to 6 months. Moreover, there is some evidence that erectile function continues to improve the longer the therapy is used.


Excitatory amino acids such as N-methyl-D,L-aspartic acid (NMDA) are thought to play an important role in the regulation of gonadotropin secretion. NMDA induces significant increases in plasma LH in a variety of animal models and these effects occur by activation of neural processes involved in excitation of LHRH neurons rather than by a direct action on the pituitary gland. We have taken advantage of this information to study the effects of NMDA on LH release and on changes in levels of LHRH mRNA in single neurons of adult rats treated neonatally with a high dosage of androgen. While iv NMDA evoked an increase in plasma LH in estrogen-treated ovariectomized control and androgen-sterilized rats (ASR), significantly less LH was released in ASR. LHRH mRNA levels in the organum vasculosum of the lamina terminalis (OVLT), the rostral (r), media (m) and caudal (c) preoptic (POA) regions were quantitated using in situ hybridization histochemistry and quantitative image analysis methods. LHRH mRNA levels in untreated controls and ASR did not differ in any of the brain regions examined. Within 1 h after NMDA, LHRH mRNA had increased significantly in OVLT and rPOA but not in mPOA and cPOA neurons of control rats and these mRNA levels remained elevated for 4 h. In contrast, NMDA treatment of ASR did not affect basal levels of LHRH mRNA in any region of the rostral hypothalamus. These observations suggest that neonatal androgen treatment of female rats either directly or indirectly affects the responsiveness of LHRH neurons to NMDA.

This study tested the hypothesis that prolactin (PRL) inhibits gonadotropin secretion in rams maintained under long days and that treatment with melatonin (s.c. continuous-release implant; MEL-IMP) reactivates the reproductive axis by suppressing PRL secretion. Adult Soay rams were maintained under long days (16L:8D) and received 1) no further treatment (control, C); 2) MEL-IMP for 16 wk and injections of saline/vehicle for the first 8 wk (M); 3) MEL-IMP for 16 wk and exogenous PRL (s.c. 5 mg ovine PRL 3x daily) for the first 8 wk (M+P). The treatment with melatonin induced a rapid increase in the blood concentrations of FSH and testosterone, rapid growth of the testes, an increase in the frequency of LH pulses, and a decrease in the LH response to N-methyl-D,L-aspartic acid. The concomitant treatment with exogenous PRL had no effect on these reproductive responses but caused a significant delay in the timing of the sexual skin color and growth of the winter pelage. These results do not support the hypothesis and suggest that PRL at physiological long-day concentrations, while being totally ineffective as an inhibitor of gonadotropin secretion, acts in the peripheral tissues and skin to maintain summer characteristics.


Phytotherapeutic agents have enjoyed widespread use, especially in Europe, for the treatment of BPH. With the recent proliferation of nutrition and vitamin stores in the United States, use of these agents has greatly increased. Although SPB extract is the most extensively studied of the phytotherapeutic agents used for BPH, no well-defined mechanism of action has been proposed. Evidence for an antiandrogenic or antiestrogenic effect is conflicting, and there are no clinical data suggesting an effect on 5-alpha-reductase activity. Furthermore, clinical trials with SPB have largely been uncontrolled and are thus of limited value in ascertaining the true clinical impact of this agent. Double-blind, controlled studies with SPB also have limitations in that most were of very short duration (none longer than 3 months) and did not provide entry or exclusion criteria. In addition, standardized symptom scores were not utilized. Only two of seven studies showed an appropriate placebo response, and the results and conclusions of both these studies were contradictory. The best and most convincing study of the efficacy of phytotherapeutic agents (using Harzol) was recently published in the Lancet. This study was rigorous and matched in design and format with pharmaceutical industry trials. A mild but appropriate placebo response was detected, which further validates the study. However, a prior placebo-controlled study showed no efficacy of beta-sitosterol-beta-D-glucoside. This dichotomy of results possibly reflects the different composition of the agents tested. This is a major confounding factor in this field of study, especially because the active ingredients are unknown. Standardization of the compounds is needed to compare and assess accurately the effect of the different extracts.

Male rhesus monkeys treated continuously with a GnRH agonist for the first 4 months of postnatal life exhibited a delay in the onset of puberty and an attenuated peripubertal rise in testosterone (T) secretion. The objectives of the current study were to determine whether these effects on sexual development were permanent and whether the hypothalamic-pituitary-testicular axis was functioning normally in these animals as adults. Neonatal GnRH agonist treatment delays but does not permanently block sexual maturation in male monkeys. Treated animals that did not show a pubertal rise in serum T during the breeding season of their 4th year exhibited a seasonal but subnormal elevation of serum T during the subsequent breeding season. Growth of the skeleton was diminished as evidenced by shorter adult crown-rump, tibia, and femur length and reduced bone mineral density of the humerus and lumbar spine. The magnitude of the serum LH and T response to iv pulses of GnRH [50 ng/kg body weight (BW)] and naloxone (1 mg/kg BW) did not differ between control and treated animals during the nonbreeding or breeding season at 6 yr of age. Conversely, treated animals showed a subnormal serum LH and T response to N-methyl-D,L-aspartic acid (5 mg/kg BW iv) during the nonbreeding season. These data suggest that adult monkeys treated neonatally with a GnRH agonist exhibit subnormal sensitivity of the central nervous system to one or more excitatory amino acids (e.g. aspartate or glutamate). Thus, abolishing neonatal activation of the pituitary-testicular axis with a GnRH agonist may permanently alter differentiation of central nervous system centers that are either involved in GnRH secretion or govern this process.


OBJECTIVES: To determine the effects of a saw palmetto herbal blend (SPHB) compared with finasteride on prostatic tissue androgen levels and to evaluate needle biopsies as a source of tissue for such determinations. METHODS: Prostate levels of testosterone and dihydrotestosterone (DHT) were measured on 5 to 10-mg biopsy specimens (18-gauge needle cores) in three groups of men with symptomatic benign prostatic hyperplasia: 15 men receiving chronic finasteride therapy versus 7 untreated controls; 4 men undergoing prostate adenomectomy to determine sampling variability (10 specimens each); and 40 men participating in a 6-month randomized trial of SPHB versus placebo, before and after treatment. RESULTS: Prostatic tissue DHT levels were found to be several times higher than the levels of testosterone (5.01 versus 1.51 ng/g), that ratio becoming reversed (1.05 versus 3.63 ng/g) with chronic finasteride therapy. The finasteride effect was statistically significant for both androgens (P <0.01), and little overlap of individual values between finasteride-treated patients was seen in the randomized trial tissue dht levels were reduced by 32% from 6 to 4 ng/g; 40% from 49 to 29 ng/g; sphb group p = 0.005 with no significant change to placebo; conclusions: for versus men androgen obtained needle biopsy specimens similar both absolute percentage change to those previously reported using surgically excised volumes prostatic quantification androgens assay biopsies feasible offers possibility serial studies sphb-induced suppression modest but a lends an element support hypothesis that inhibition enzyme 5-alpha reductase mechanism action this substance

OBJECTIVE: To establish an etiological diagnosis in two unrelated Egyptian children with ambiguous genitalia through biochemical and molecular analyses. PATIENTS AND METHODS: Two XY patients were referred: one at the age of 14 years presenting with delayed puberty and menarche and the second at the age of 4 months with ambiguous genitalia. Basal and post-HCG stimulation plasma levels of testosterone (T) and dihydrotestosterone (DHT) were determined. Direct sequencing of the five exons of the 5alphaR type 2 gene and exons 2 to 8 of the androgen receptor gene was carried out. RESULTS: The high T/DHT value indicated 5alphaR deficiency in the first patient while the absence of parental consanguinity along with normal T/DHT value in the second patient suggested androgen insensitivity. In both patients, we identified a homozygous A --> G mutation in exon 3 that replaced the asparagine residue at position 160 by an aspartic acid. The parents of both patients were all heterozygotes for the N160D substitution. CONCLUSIONS: 1) We report a new mutation that enlarges the spectrum of genetic defects in 5alphaR deficiency. 2) Although the two patients were referred at very different ages, the clinical presentations raise the possibility of phenotypic variability for the same mutation. 3) These reports underline the difficulty of diagnosing 5alphaR deficiency based only on clinical and biochemical grounds. Molecular study remains the only definitive tool for diagnosis of ambiguous genitalia.


As brain testosterone plays both androgenic and estrogenic actions due to its conversion into estrogen via aromatase naturally, it is unclear that the age-related reduction of testosterone increased risk of Alzheimer's disease (AD) in men is mediated through androgen alone or both androgen and estrogen mechanisms. Our previous studies using a gene-based approach in mouse model to block the conversion of testosterone into estrogen (aromatase gene knock-out, ArKO), found a depletion of estrogen and increase in testosterone endogenously in males. Here, we use crossing the ArKO mice with APP23 transgenic mice, a mouse model of AD, to produce APP23/Ar(+/−) mice to study the estrogen-independent effect of testosterone on AD. We found a significant reduction in brain plaque formation, improved cognitive function and increase NEP activity in male APP23/Ar(+/−) mice compared with age-matched male APP23 controls. In addition, we found, for the first time, a reduction of beta-secretase (BACE1) enzyme activity, mRNA level and protein expression in the male APP23/Ar(+/-) mice, suggesting that endogenous testosterone, independent from estrogen, may protect against AD in males via two major mechanisms, downregulation of BACE1 activities at transcriptional level to reduce beta amyloid production and upregulation of NEP activities to enhance bate amyloid degradation.

McKeever, B. M., B. K. Hawkins, et al. (2002). "Amino acid substitution of arginine 80 in 17beta-hydroxysteroid dehydrogenase type 3 and its effect on NADPH cofactor binding and
17beta-Hydroxysteroid dehydrogenase type 3 (17beta-HSD-3) is a member of the short-chain dehydrogenase/reductase (SDR) family and is essential for the reductive conversion of inactive C(19)-steroid, androstenedione, to the biologically active androgen, testosterone, which plays a central role in the development of the male phenotype. Mutations that inactivate this enzyme give rise to a rare form of male pseudohermaphroditism, referred to as 17beta-HSD-3 deficiency. One such mutation is the replacement of arginine at position 80 with glutamine, compromising enzyme activity by increasing the cofactor binding constant 60-fold. In the absence of a 17beta-HSD-3 crystal structure, we have grafted its amino acid sequence for the NADPH binding site on the X-ray crystal structures of glutathione reductase (Protein Data Bank code 1gra) and 17beta-HSD type 1 (Protein Data Bank codes 1fdv and 1fdu) where we find the trunk of the arginine 80 side chain forms part of the hydrophobic pocket for the purine ring of adenosine while its guanidinium moiety interacts with the 2'-phosphate to both stabilize cofactor binding and neutralize its intrinsic negative charge through two hydrogen bonds. To qualitatively assess the role arginine 80 plays in both selecting and stabilizing NADPH binding, it was replaced with each amino acid and the mutant enzymes subjected to enzymatic analysis. There are only seven enzymes exhibiting any measurable enzymatic activity with arginine approximately lysine>leucine>glutamine>methionine>tyrosine>Isoleucine. With an aspartic acid at position 58 in 17beta-HSD-3 occupying the equivalent space in the cofactor binding pocket as arginine 224 in glutathione reductase or serine 12 in 17beta-HSD-1, there was an expectation that some of the mutants might use NADH as a cofactor. In no case was NADH found to substitute for NADPH.


INTRODUCTION: Lower urinary tract symptoms related to benign prostatic hyperplasia (BPH) and bladder outlet obstruction may affect up to 30% of men in their early 70s. Symptoms can improve without treatment, but the usual course is a slow progression of symptoms, with acute urinary retention occurring in 1% to 2% of men with BPH per year. METHODS AND OUTCOMES: We conducted a systematic review and aimed to answer the following clinical questions: What are the effects of medical, herbal, and surgical treatments? We searched: Medline, Embase, The Cochrane Library, and other important databases up to July 2009 (Clinical Evidence reviews are updated periodically, please check our website for the most up-to-date version of this review). We included harms alerts from relevant organisations such as the US Food and Drug Administration (FDA) and the UK Medicines and Healthcare products Regulatory Agency (MHRA). RESULTS: We found 63 systematic reviews, RCTs, or observational studies that met our inclusion criteria. We performed a GRADE evaluation of the quality of evidence for interventions. CONCLUSIONS: In this systematic review we present information relating to the effectiveness and safety of the following interventions: 5 alpha-reductase inhibitors, alpha-blockers, beta-sitosterol plant extract, Pygeum africanum, rye grass pollen extract, saw palmetto plant extracts, transurethral electrovaporisation, transurethral Holmium laser enucleation of the prostate, transurethral microwave thermotherapy, transurethral needle ablation, and transurethral resection (including transurethral resection versus transurethral incision, and transurethral resection versus visual laser ablation/laser vaporisation).

Oestrogens have neuroprotective properties, resulting in memory and learning preservation. Red wine (RW) has been linked to neuroprotection, but mechanisms are largely unknown. The aim of this work was to test the effect of RW or 13% ethanol solution consumption on the expression of aromatase and estrogen receptors (ER) in the rat hippocampus. Beverages were supplied to male Wistar rats and after 8 weeks of treatment animals were euthanised, hippocampus was removed, aromatase expression assessed by western blotting and aromatase and ER transcription determined by RT-PCR. The effects of treatments on hippocampal aromatase activity were also determined, as well as the effect of several red wine polyphenols in hippocampal homogenates from untreated animals. Aromatase transcription was increased by ethanol (to 158+/−7%) but only significantly by RW (to 180+/−9%). No difference was found in ERalpha expression among groups, whereas RW significantly decreased ERbeta expression (to 63+/−10%). Resveratrol, quercetin, myricetin and kaempferol had no effect on aromatase activity and catechin (300 microM), epicatechin (200 microM), procyanidin extract (200 mg/L) and fractioned procyanidins (F1 and FII; 200 mg/L) significantly decreased aromatase activity. The contribution of procyanidins in wine to the effect observed in aromatase was investigated in animals treated for the same period with these compounds (200 mg/L), although no effect was seen in aromatase activity, mRNA or protein levels, meaning that this group of compounds had little contribution, if any, to the effects observed. Nevertheless, the increase in aromatase expression induced by RW may corroborate the neuroprotective ability attributed to this beverage. Alterations in the relative abundance of ER expression may also play an important role in the protection.


Flavonoids are present in fruits, vegetables and beverages derived from plants (tea, red wine), and in many dietary supplements or herbal remedies including Ginkgo Biloba, Soy Isoflavones, and Milk Thistle. Flavonoids have been described as health-promoting, disease-preventing dietary supplements, and have activity as cancer preventive agents. Additionally, they are extremely safe and associated with low toxicity, making them excellent candidates for chemopreventive agents. The cancer protective effects of flavonoids have been attributed to a wide variety of mechanisms, including modulating enzyme activities resulting in the decreased carcinogenicity of xenobiotics. This review focuses on the flavonoid effects on cytochrome P450 (CYP) enzymes involved in the activation of procarcinogens and phase II enzymes, largely responsible for the detoxification of carcinogens. A number of naturally occurring flavonoids have been shown to modulate the CYP450 system, including the induction of specific CYP isozymes, and the activation or inhibition of these enzymes. Some flavonoids alter CYPs through binding to the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, acting as either AhR agonists or antagonists. Inhibition of CYP enzymes, including CYP 1A1, 1A2, 2E1 and 3A4 by competitive or mechanism-based mechanisms also occurs. Flavones (chrysin, baicalein, and galangin), flavanones (naringenin) and isoflavones (genistein, biochanin A) inhibit the activity of aromatase (CYP19), thus decreasing estrogen biosynthesis and producing antiestrogenic effects, important in breast and prostate cancers. Activation of phase II detoxifying enzymes, such as UDP-glucuronyl transferase, glutathione S-transferase, and quinone reductase by flavonoids results in the detoxification of carcinogens and represents one mechanism of their anticarcinogenic effects. A number of flavonoids including fisetin, galangin, quercetin, kaempferol, and genistein represent potent non-competitive inhibitors of sulfotransferase 1A1 (or P-PST); this may represent an important mechanism for the chemoprevention of
sulfation-induced carcinogenesis. Importantly, the effects of flavonoids on enzymes are generally dependent on the concentrations of flavonoids present, and the different flavonoids ingested. Due to the low oral bioavailability of many flavonoids, the concentrations achieved in vivo following dietary administration tend to be low, and may not reflect the concentrations tested under in vitro conditions; however, this may not be true following the ingestion of herbal preparations when much higher plasma concentrations may be obtained. Effects will also vary with the tissue distribution of enzymes, and with the species used in testing since differences between species in enzyme activities also can be substantial. Additionally, in humans, marked interindividual variability in drug-metabolizing enzymes occurs as a result of genetic and environmental factors. This variability in xenobiotic metabolizing enzymes and the effect of flavonoid ingestion on enzyme expression and activity can contribute to the varying susceptibility different individuals have to diseases such as cancer. As well, flavonoids may also interact with chemotherapeutic drugs used in cancer treatment through the induction or inhibition of their metabolism.


Activation of the alpha(2)-adrenoceptor has been shown to produce antinociception. We have previously shown that the antinociceptive effect of clonidine, an alpha(2)-adrenoceptor agonist, is sex-specific and is abolished by exogenous estrogen in ovariectomized rats or high level of endogenous estrogen in proestrous females. Here, we investigated whether testosterone mediates the antinociceptive effect of clonidine in the trigeminal region of the male rat. Clonidine (7 microg/5 microl) was injected intracisternally through a PE-10 cannula implanted dorsal to the trigeminal region in orchidectomized (GDX) male Sprague-Dawley rats. In separate groups, testosterone propionate (250 microg/100 microl; GDX+T) or beta-estradiol benzoate (100 microg/100 microl; GDX+E) were injected subcutaneously 24 and 48 h respectively prior to the N-methyl-D-aspartic acid (NMDA)--or heat-evoked nociceptive test. NMDA-induced number of scratches or duration of scratching behavior did not change significantly in control groups with or without hormonal replacement. Clonidine significantly reduced both measures only in the GDX+T group but not in GDX or GDX+E group. Clonidine also significantly increased head withdrawal latency (HWL) in the GDX+T group, but not in GDX or GDX+E group. The antinociceptive effect of clonidine was reversed by yohimbine, an alpha(2)-adrenoceptor antagonist, in GDX+T group. We conclude that testosterone is required for the expression of antinociception produced by selective activation of the alpha(2)-adrenoceptor in the trigeminal region of the male rat. These findings further our understanding of sex-related differences in the modulation of nociception and may provide insight into development and administration of analgesic agents in young vs. aging men.


D-Aspartate increases human chorionic gonadotropin-induced testosterone production in purified rat Leydig cells. L-Aspartate, D-,L-glutamate or D-,L-asparagine could not substitute for D-aspartate and this effect was independent of glutamate receptor activation. Testosterone production was enhanced only in cells cultured with D-aspartate for more than 3 h. The increased production of testosterone was well correlated with the amounts of D-aspartate incorporated into the Leydig cells, and L-cysteine sulfenic acid, an inhibitor of D-aspartate uptake, suppressed both testosterone production and intracellular D-aspartate
levels. D-Aspartate therefore is presumably taken up into cells to increase steroidogenesis. Intracellular D-aspartate probably acts on cholesterol translocation into the inner mitochondrial membrane, the rate-limiting process in steroidogenesis.


D-aspartate and human chorionic gonadotropin act synergistically to increase testosterone production in purified rat Leydig cells, and D-aspartate stimulates testosterone synthesis even in the absence of human chorionic gonadotropin stimulation. In addition, D-aspartate enhances steady-state cellular mRNA and protein levels of steroidogenic acute regulatory protein, which is a key regulatory factor in gonadal and adrenal steroidogenesis. D-aspartate therefore appears to increase testosterone production in rat Leydig cells by stimulating steroidogenic acute regulatory protein gene expression. To our knowledge, this is the first report demonstrating a direct effect of D-aspartate on gene expression in mammalian cells.


The present study investigated the effects of stinging nettle (Urtica dioica L.) (UD) on benign prostatic hyperplasia (BPH) induced by testosterone. In vitro studies were conducted to assess the 5alpha-reductase inhibitory potential of UD. Two biochemical markers viz., beta-sitosterol and scopoletin, were isolated and characterised in the extracts utilising High-performance thin layer chromatographic, FTIR, NMR and overlain UV spectral studies. Hyperplasia was induced in rats by subcutaneous administration of testosterone (3 mg kg(-1) s.c.) for 28 days in all the groups except the vehicle-treated group. Simultaneous administration of petroleum ether and ethanolic extracts (10, 20 and 50 mg kg(-1) p.o.) and isolated beta-sitosterol (10 and 20 mg kg(-1) p.o.) was undertaken. Finasteride was used as a positive control (1 mg kg(-1) p.o.). Measurement of prostate/body weight ratio, weekly urine output and serum testosterone levels, prostate-specific antigen levels (on day 28) and histological examinations carried out on prostates from each group led us to conclude that UD can be used as an effective drug for the management of BPH.


The aim of the present study was to find out whether Ganoderma lucidum (GL) can be used as a clinically effective medicine for the management of prostatic hyperplasia. In vitro studies were conducted to assess the 5alpha-reductase inhibitory potential of GL. A biochemical marker viz. beta-sitosterol was identified and characterised in the extracts utilising high-performance thin-layer chromatography. Testosterone (3 mg kg(-1) s.c.) was administered to the rats along with the test extracts (10, 20 and 50 mg kg(-1) p.o.) and beta-sitosterol (10 and 20 mg kg(-1) p.o.) for a period of 28 days. Finasteride was used as a positive control (1 mg kg(-1) p.o.). GL extracts attenuated the increase in the prostate/body weight ratio induced by testosterone. Petroleum ether extract exhibiting the best activity. Ethanolic extract also exhibited
significant activity. The urine output also improved significantly, which emphasises the clinical implications of the study. Testosterone levels measured weekly and prostate-specific antigen (PSA) levels measured at the end of the study also support our claims. The PSA levels decreased in the extract-treated groups, indicating their usefulness in the treatment of benign prostatic hyperplasia. Histological studies have shown a considerable improvement in the prostatic histoarchitecture in the extract-treated groups when compared to the testosterone-treated group.


The present study undertook chemical analysis of components of Pfaffia paniculata roots. In addition, an animal experiment was conducted in which mice had ad libitum access to water enriched with powdered P. paniculata root for 30 days. Changes in plasma concentrations of estradiol-17beta and progesterone in female mice and of testosterone in male mice were ascertained. The results revealed that P. paniculata roots contain two types of phytosteroids, beta-sitosterol and stigmasterol, in addition to other compounds such as pfaffic acid, allantoin, saponins, beta-sitosteryl-beta-D-glucoside, and stigmasteryl-beta-D-glucoside. Regarding changes in plasma concentrations of hormones, levels of the sex hormones estradiol-17beta, progesterone and testosterone were clearly higher for mice that drank P. paniculata root-enriched water than for mice that drank plain water. Powdered P. paniculata root is easily dissolved in feed or water, and as no adverse reactions were seen in mice within 30 days of oral intake, consumption of P. paniculata for long periods of time appears safe.


INTRODUCTION: The nicotinamide adenine dinucleotide phosphate (NADPH)-dependent membrane protein 5alpha-reductase irreversibly catalyses the conversion of testosterone to the most potent androgen, 5alpha-dihydrotestosterone (DHT). In humans, two 5alpha-reductase isoenzymes are expressed: type I and type II. Type II is found primarily in prostate tissue. Saw palmetto extract (SPE) has been widely used for the treatment of lower urinary tract symptoms secondary to benign prostatic hyperplasia (BPH). The mechanisms of the pharmacological effects of SPE include the inhibition of 5alpha-reductase, among other actions. Clinical studies of SPE have been equivocal, with some showing significant results and others not. These inconsistent results may be due, in part, to varying bioactivities of the SPE used in the studies. METHODS: The aim of the present study was to determine the in vitro potency of a novel saw palmetto ethanol extract (SPET-085), an inhibitor of the 5alpha-reductase isoenzyme type II, in a cell-free test system. On the basis of the enzymatic conversion of the substrate androstenedione to the 5alpha-reduced product 5alpha-androstanedione, the inhibitory potency was measured and compared to those of finasteride, an approved 5alpha-reductase inhibitor. RESULTS: SPET-
085 concentration-dependently inhibited 5alpha-reductase type II in vitro (IC(50)=2.88±0.45 microg/mL). The approved 5alpha-reductase inhibitor, finasteride, tested as positive control, led to 61% inhibition of 5alpha-reductase type II. CONCLUSION: SPET-085 effectively inhibits the enzyme that has been linked to BPH, and the amount of extract required for activity is very low compared to data reported for other extracts. It can be concluded from data in the literature that SPET-085 is as effective as a hexane extract of saw palmetto that exhibited the highest levels of bioactivity, and is more effective than other SPEs tested. This study confirmed that SPET-085 has prostate health-promoting bioactivity that also corresponds favorably to that reported for the established prescription drug standard of therapy, finasteride.


Since D-aspartate stimulates prolactin and LH release, our objective was to determine whether D-aspartate modifies the release of hypothalamic and posterior pituitary factors involved in the control of their secretion and whether its effects on these tissues are exerted through NMDA receptors and mediated by nitric oxide. In the hypothalamus, D-aspartate stimulated luteinizing hormone-releasing hormone (LHRH), alpha-melanocyte-stimulating hormone (alpha-MSH) and GABA release and inhibited dopamine release through interaction with NMDA receptors. It increased nitric oxide synthase (NOS) activity, and its effects on LHRH and hypothalamic GABA release were blunted when NOS was inhibited. In the posterior pituitary gland, D-aspartate inhibited GABA release but had no effect on dopamine or alpha-MSH release. We report that D-aspartate differentially affects the release of hypothalamic and posterior pituitary factors involved in the regulation of pituitary hormone secretion.


Among medicinal plants, extract from the hollyhock flowers is a source of antocyanides and flavonoids. The latter compounds belong, among others, to phytoestrogens (plant-derived dietary estrogens). The important role of estrogens in the testis is now well documented, and phytoestrogens, which may act as estrogen agonists or estrogen antagonists can also alter the reproductive function of the male. The aim of this study was to show whether the exposure of male rats to the aqueous hollyhock extract could affect the process of aromatization in their testes and in cultured Leydig cells. This was investigated by immunocytochemistry and radioimmunological assays. Immunoreactivities for aromatase and estrogen receptor beta were weaker both in testicular sections and cultured Leydig cells after hollyhock extract administration when compared to the controls, while the intensity of immunoreaction for estrogen receptor alpha remained unchanged. A lower level of estradiol secreted by cultured Leydig cells from the experimental group positively correlated with a direct inhibition of aromatase activity. Additionally, a quantitative analysis of flavonoid fraction from the hollyhock extract revealed the presence of quercetin and kaempferol. It seems that a weak antiestrogenic activity of flavonoid compounds present in the hollyhock extract is mediated through aromatase and estrogen receptor beta rather than by estrogen receptor alpha.


In the study, the inhibitory effect of flavonoids, including isoflavonic phytoestrogens, on the ovarian aromatase enzyme complex from the rainbow trout, Oncorhynchus mykiss, was assessed in vitro. Some of the compounds tested on fish were also tested on human placental aromatase activity as a comparison between the two sources of enzyme. It was found that flavone, dl-aminogluthethimide, apigenin, quercetin, 7,4'-dihydroxyflavone, alpha-naphthoflavone and equol were potent inhibitors of the ovarian aromatase activity in rainbow trout. Relative potencies (RP) of these compounds compared to flavone (assigned an effect of 1) were, respectively, 19.0, 8.7, 5.3, 3.7, 3.2 and 0.9. Two other phytoestrogens, namely biochanin A and genistein, slightly inhibited aromatase activity. Finally, 7-hydroxyflavone, formononetin, daidzein, coumestrol, chrysin, flavanone and estradiol-17beta did not inhibit ovarian aromatase activity at doses up to 1000 microM. Experiments on human placental aromatase showed inhibitory effects of dl-aminogluthethimide, flavone, flavanone and equol with RP values of 2.8, 1.1, 1.5 and 0.4, respectively. These results are in accordance with previous studies. The influence of the experimental procedure on IC50 values and RP is discussed.


Activation of N-methyl-D,L-aspartic acid (NMDA) receptors stimulates growth hormone (GH) secretion. The mechanisms involved in this action are still a matter of debate. Present experiments were carried out to assess specifically: (1) the age-related changes in NMDA effects; (2) the physiological role of NMDA in pulsatile GH secretion; (3) the hypothalamic and/or pituitary actions of NMDA, and (4) the influence of gonadal function on NMDA-induced GH release. NMDA (15 mg/kg i.p.) stimulated GH secretion in neonatal, prepubertal and adult males, this effect being blocked by MK-801, a selective antagonist of NMDA receptors. In adult males, pulsatile GH secretion was abolished after administration of MK-801 and AP-5, antagonists of NMDA receptors. The stimulatory effect of NMDA on GH release was exerted at the hypothalamic level, since in vitro GH secretion was slightly inhibited in the presence of NMDA (0.5 mM). The increase in GH release after NMDA treatment cannot be explained through an increase in GHRH release, as the NMDA effect persisted in animals pretreated with GHRH antisemum and in those neonatally injected with mono- sodium glutamate, a drug that destroys GHRH neurons. In addition, NMDA-induced GH secretion was independent of testicular function since it remained after orchidectomy, testosterone replacement as well as after permanent damage of testicular function by neonatal administration of estrogens (500 microg on day 1 of life). We conclude that NMDA receptors play a physiological role stimulating GH secretion through a hypothalamic mechanism that is, at least partially, not GHRH-dependent, and is not modulated by testicular secretion.


It is well known that in the rat neonatal manipulation of the sex steroid environment results in altered hypothalamic-pituitary function in adulthood which implies an abnormal prolactin secretion and gonadotrophin response to orchidectomy. The present paper analyses the involvement of excitatory amino acid pathways in the disturbed gonadotrophin and prolactin secretion in male rats neonatally injected with oestradiol benzoate (500 micrograms on the day of birth). In the first experiment, 60-day-old control and oestrogenized male rats (intact or orchidectomized a week before) were killed 15 min after injection of vehicle, N-methyl-D-aspartic acid (NMDA; 15 mg/kg) or kainic acid (KA; 15 mg/kg). In the second experiment, prepubertal males were killed 15 min after injection of vehicle or NMDA. In the third experiment 30-, 45-, 60- and 90-day-old intact control and oestrogenized males were killed 15 min after injection of vehicle or KA. In the fourth experiment, control and oestrogenized males were sham-orchidectomized, orchidectomized or orchidectomized and implanted with Silastic capsules at 30 days of age and killed on day 90, 15 min after vehicle or KA injection.(ABSTRACT TRUNCATED AT 250 WORDS)


Administration of sex steroids to neonatal female rats resulted in anovulation and absence of positive and negative feedback between oestradiol and LH secretion. In the present experiments, the role of excitatory amino acids in the control of gonadotrophin secretion in anovulatory adult rats sterilized by neonatal administration of oestradiol benzoate or testosterone propionate (100 mg or 1.25 mg on the day of birth, respectively) was studied. Cyclic females in metoestrus were used as controls. Serum LH and FSH concentrations were measured at different times after i.p. administration of N-methyl-D-aspartic acid (NMDA), kainic acid (agonists of NMDA and kainate receptors, respectively), MK-801 or 6,7-dinitroquinoxaline-2,3-dione (DNQX) (antagonists of NMDA and kainate receptors, respectively). Experiments were also performed in control and sterilized females 1 week after ovariectomy. It was found that: (1) the effectiveness of NMDA and kainic acid in stimulating LH secretion was significantly higher in sterilized than in cyclic females; (2) ovariectomy increased LH secretion only in control females; (3) the stimulatory effect of NMDA and kainic acid on LH secretion after ovariectomy was observed only in sterilized females; (4) MK-801 and DNQX selectively decreased LH secretion in sterilized females; and (5) FSH secretion remained unaffected after NMDA or kainic acid administration in both control and sterilized females. In conclusion, the results obtained in sterilized females showed both a tonic release of endogenous excitatory amino acids and a greater responsiveness to NMDA and kainic acid than in controls.


OBJECTIVE: The stimulatory and inhibitory effects of N-methyl-D-aspartic acid (NMDA) and kainic acid on
Prolactin (PRL) secretion have been correlated with the serum prolactin concentrations before drug administration. In the present experiments, we analysed the role of NMDA and kainic acid in PRL secretion in females with different serum concentrations of PRL. METHODS: Hypoprolactinaemic females were obtained by ovariectomy or after administration of diethyldithiocarbamate (an inhibitor of dopamine-beta-hydroxylase). Chronic hyperprolactinaemia was induced by neonatal administration of testosterone or oestradiol and acute hyperprolactinaemia was induced either by administration of alpha-methyl-p-tyrosine (an inhibitor of tyrosine hydroxylase) or by ether exposure. To analyse the role of dopamine in the effects of NMDA, we measured pituitary concentrations of dopamine after NMDA treatment and the effects of pretreatment with domperidone. RESULTS: (1) NMDA, but not kainic acid, stimulated PRL release in cyclic females. This effect was independent of serum PRL concentrations and was not accompanied by a decrease in pituitary concentrations of dopamine. (2) NMDA did not change PRL secretion in neonatally androgenized females, whereas NMDA and kainic acid inhibited PRL release in neonatally oestrogenized females. The inhibitory effects of NMDA and kainic acid were blocked by domperidone. (3) Kainic acid inhibited PRL secretion in prepubertal hyper- and hypoprolactinaemic rats. (4) Hyperprolactinaemia induced by ether stress was counteracted by administration of NMDA and kainic acid. CONCLUSIONS: (a) NMDA has a dual effect on prolactin secretion that is independent of prior prolactin concentrations and of dopamine activity, but kainic acid is only inhibitory. (b) The stimulatory or inhibitory effects of NMDA and kainic acid on PRL secretion were not strictly related to basal PRL concentrations and necessarily involved a change in the secretion of prolactin releasing factors, as no correlations were observed between changes in pituitary concentrations of dopamine and serum PRL concentrations. (c) Females rendered hyperprolactinaemic by neonatal administration of testosterone or oestradiol responded differently after NMDA administration. (d) NMDA and kainic acid blocked the mechanisms involved in stress-induced PRL secretion.


It is well known that the control of LH secretion depends on the steroid milieu during the postnatal period. In this study LH secretion was analysed in adult male rats injected neonatally with 500 micrograms oestradiol benzoate (1) after orchidectomy, (2) after selective elimination of androgens by destruction of Leydig cells with ethylene dimethane sulphonate (EDS), and (3) after removal in orchidectomized animals of Silastic capsules containing testosterone. In addition, (4) in vivo and in vitro LH secretion in response to LHRH agonist and antagonists, (5) the hypothalamic LHRH content, (6) the basal and stimulated in vitro LHRH release, and (7) the LH responses after administration of naloxone (2 mg/kg), alpha-methyl-p-tyrosine (alpha-MPT; 250 mg/kg), N-methyl-D-aspartic acid (NMNDA, 15 mg/kg) or kainic acid (KA; 15 mg/kg) were also examined. Our data indicated that (1) the LH response after orchidectomy, after EDS administration and after removal of Silastic capsules containing testosterone was diminished in oestrogenized male rats, (2) the pituitaries from oestrogenized males retained responsiveness to LHRH, (3) hypothalamic LHRH content was reduced in oestrogenized males, but the hypothalamus from oestrogenized males released more LHRH than those of control groups both under basal conditions or after depolarization, (4) alpha-MPT decreased LH secretion only in oestrogenized males, and (5) NMNDA and KA stimulated LH only in oestrogenized males. We conclude that in oestrogenized male rates the loss of sensitivity to the negative feedback action of testosterone on LH secretion was not due to decreased pituitary responsiveness to LHRH stimulation or to the inherent damage of LHRH neurones.(ABSTRACT TRUNCATED AT 250 WORDS)


Endogenous sex hormones have been observed to have a role in systemic lupus erythematosus (SLE) predisposition. Sex hormone-binding globulin (SHBG) regulates the bioavailability of sex hormones to target tissues. Therefore, we examined the distribution of the SHBG functional polymorphism Asp327Asn (rs6259) in SLE patients (n = 150) and controls (n = 150) in a Polish population. We found a contribution of the SHBG327Asn variant to the development of SLE. Women with the Asp/Asn and Asn/Asn genotypes displayed a 2.630-fold increased risk of SLE (95% CI = 1.561-4.433, P = 0.0003). SHBG has a much higher affinity for testosterone than estradiol, and the SHBG327Asn variant displays a reduction of estradiol clearance. Therefore we suggest that the opposing effects of estrogens and testosterone on the immune system and imbalance in the levels of these hormones in SLE patients can be enhanced by the SHBG327Asn protein variant.


BACKGROUND: Androgenetic alopecia (AGA) is characterized by the structural miniaturization of androgen-sensitive hair follicles in susceptible individuals and is anatomically defined within a given pattern of the scalp. Biochemically, one contributing factor of this disorder is the conversion of testosterone (T) to dihydrotestosterone (DHT) via the enzyme 5-alpha reductase (5AR). This metabolism is also key to the onset and progression of benign prostatic hyperplasia (BPH). Furthermore, AGA has also been shown to be responsive to drugs and agents used to treat BPH. Of note, certain botanical compounds have previously demonstrated efficacy against BPH. Here, we report the first example of a placebo-controlled, double-blind study undertaken in order to examine the benefit of these botanical substances in the treatment of AGA. OBJECTIVES: The goal of this study was to test botanically derived 5AR inhibitors, specifically the liposterolic extract of Serenoa repens (LSESr) and beta-sitosterol, in the treatment of AGA. Subjects: Included in this study were males between the ages of 23 and 64 years of age, in good health, with mild to moderate AGA. RESULTS: The results of this pilot study showed a highly positive response to treatment. The blinded investigative staff assessment report showed that 60% of (6/10) study subjects dosed with the active study formulation were rated as improved at the final visit. CONCLUSIONS: This study establishes the effectiveness of naturally occurring 5AR inhibitors against AGA for the first time, and justifies the expansion to larger trials.


This study investigated the involvement of D-aspartic acid (D-Asp) in testicular steroidogenesis of the green frog Rana esculenta and its effect on stimulation of thumb pad morphology and glandular activity, a typical testosterone-dependent secondary sexual characteristic in this amphibian species. In the testis, D-Asp concentrations vary significantly during the reproductive cycle: they are low in pre- and post-
reproductive periods, but reach peak levels in the reproductive period (140-236 nmol/g wet tissue). Moreover, the concentrations of D-Asp in the testis through the sexual cycle positively match the testosterone levels in the gonad and the plasma. The racemase activity evaluated during the cycle expresses its peak when D-Asp and testosterone levels are highest, that is, during the reproductive period, confirming the synthesis of D-Asp from L-Asp by an aspartate racemase. Short-term in vivo experiments consisting of a single injection of D-Asp (2.0 micro mol/g body weight) demonstrated that this amino acid accumulates significantly in the testis, and after 3 h its uptake is coupled with a testosterone increase in both testis and plasma. Moreover, within 18 h of amino acid administration, the D-Asp concentration in the testis decreased along with the testosterone titer to prestimulation levels. Other amino acids (L-Asp, D-Glu and L-Glu) used instead of D-Asp were ineffective, confirming that the significant increase in testicular testosterone was a specific feature of this amino acid. In long-term experiments, D-Asp had been administered chronically to frogs caught during the three phases of the reproductive cycle, inducing testosterone increase and 17beta-estradiol decrease in the gonad during the pre- and post-reproductive period, and vice versa during the reproductive period. The stimulatory effect of D-Asp on testosterone production by the testis is consistent with the stimulation of spermatogenesis and the maturation of thumb pads occurring in D-Asp-treated frogs. In these last animals, there was an increase of seminiferous ampoule area and a higher number of spermatids and sperm. Moreover, in spermatogonia I and II and in spermatocytes, a proliferating cell nuclear antigen (PCNA) intense immunopositivity was observed. In addition, the thumb pads of D-Asp-treated frogs compared with controls showed a significantly thicker epithelial lining, a wider area of their glands with taller secretion cells, and more numerous, PAS-positive-rich secretions. Finally, these results provide functional evidence for a biologic role of D-Asp in amphibian male steroidogenesis; therefore, this unusual amino acid could be considered a modulatory agent for reproductive processes.


In the lizard Podarcis s. sicula, a substantial amount of D-aspartate (D-Asp) is endogenous to the testis and shows cyclic changes of activity connected with sex hormone profiles during the annual reproductive phases. Testicular D-Asp content shows a direct correlation with testosterone titres and a reverse correlation with 17beta-estradiol titres. In vivo experiments, consisting of i.p. injections of 2.0 micromol/g body weight of D-Asp or other amino acids, in lizards collected during the three main phases of the reproductive cycle (pre-reproductive, reproductive and post-reproductive period), revealed that the testis can specifically take up and accumulate D-Asp alone. Moreover, this amino acid influences the synthesis of testosterone and 17beta-estradiol in all phases of the cycle. This phenomenon is particularly evident during the pre- and post-reproductive period, when endogenous testosterone levels observed in both testis and plasma were the lowest and 17beta-estradiol concentrations were the highest. D-Asp rapidly induces a fall in 17beta-estradiol and a rise in testosterone at 3 h post-injection in the testis and at 6 h post-injection in the blood. In vitro experiments show that testicular tissue converted L-Asp into D-Asp through an aspartate racemase. D-Asp synthesis was measured in all phases of the cycle, but was significantly higher during the reproductive period with a peak at pH 6.0. The exogenous D-Asp also induces a significant increase in the mitotic activity of the testis at 3 h (P < 0.05) and at 6 h (P < 0.01). Induction of spermatogenesis by D-Asp is recognized by an intense immunoreactivity of the germinal epithelium (spermatogonia and spermatids) for proliferation cell nuclear antigen (PCNA). The effects of D-Asp on the testis appear to be specific since they were not seen in lizards injected with other D- or L-forms of amino acids with known excitatory effects on neurosecretion. Our results suggest a regulatory role for D-Asp in the steroido-genesis and spermatogenesis of the testis of the lizard Podarcis s. sicula.

The current study provides substantial evidence that the pattern of synthesis of D-aspartic acid (D-Asp) in the testes of lizard Podarcis s. sicula throughout the reproductive cycle is in parallel with seasonal variations of testosterone, c-kit receptor protein, tyrosine kinase activity, and proliferating cell nuclear antigen (PCNA) protein. Although the trend is the same in all phases of the sexual cycle, the peaks of these three molecules are detectable only during the reproductive period. Using Western blot technique, we demonstrated that both polyclonal c-kit and PCNA antibodies specifically recognized bands with molecular mass of approximately 150 and approximately 36 kDa, respectively. By immunocytochemical methods, D-Asp immunopositivity appeared spread in the germinal epithelium as well as in the interstitial compartment of the testes. We also found specific c-kit labeling in I and II spermatogonia (SPG), in I and II spermatocytes (SPC), in the elongated spermatides, in spermatozoa, in Sertoli and Leydig cells. Like c-kit, PCNA positivity was located in the germinal epithelium pattern. Furthermore, we investigated the relationship between testosterone, c-kit receptor, tyrosine kinases activity and PCNA following treatment with D-Asp. In vivo experiments, entailing a single injection of D-Asp (2.0 micromol/g body weight), demonstrated that this amino acid significantly accumulated in the testes. After 3 h, its uptake was accompanied by an increase in testosterone levels and in the expression and intensity of immunostaining of c-kit receptor protein. Furthermore, at 6 h, exogenous D-Asp affected the phosphorylation of tyrosine kinases, whose activation was positively correlated with the temporal uptake of both D-Asp and testosterone detected in the testes. Thereafter, between 6 and 15 h, the expression of PCNA was induced and an increase in its immunolabeling intensity was observed. Taken all together, these results provide new insights into the testicular activity during the reproductive cycle of Podarcis s. sicula, suggesting that a sequential cascade of a functional relationship between testosterone levels, c-kit receptor protein, tyrosine kinase activity and PCNA could be partly mediated by D-aspartic acid.


We investigated whether the maturation of oocyte follicular epithelium of lizard is affected by d-aspartic acid (d-Asp). Our results demonstrated that d-Asp is endogenously present in the oocytes, and its distribution varies during the reproductive cycle and following intraperitoneal administration. At previtellogenesis, it is observed in the cytoplasm and nucleus of pyriform cells, in intermediate cells, in some small cells of the granulosa, in the ooplasm, and in some thecal elements. At vitellogenesis, d-Asp is localized in the proximity of the zona pellucida, in the theca, and in the ooplasm. Injected d-Asp is mainly captured by pyriform cells and ooplasm of previtellogenic oocytes, but a moderate accumulation is evident in the cytoplasm of some small granulosa cells and in the theca. d-Asp also increases the ovarian and plasmatic levels of 17beta-estradiol and decreases those of testosterone. As a direct and/or indirect consequence of d-Asp, previtellogenic oocytes grow up and mature, resulting in a higher accumulation of carbohydrates in the granulosa, zona pellucida, and ooplasm, but also a reduction in the thickness of the granulosa layer and an increase of the theca stratum. Taken together, our results show that d-Asp may be related to the synchrony of reproduction, either enhancing the growth and maturation of follicular epithelium or influencing its endocrine functions. (J Histochem Cytochem 58:157-171, 2010).
We investigated the involvement of D-Aspartic acid (D-Asp) on ovarian and testicular morphology of the green frog, Rana esculenta, and its effect on the testosterone production. The study has been performed throughout the reproductive cycle. In both ovary and testis a substantial amount of D-Asp is endogenously present and its concentration varies as function of reproduction. In the frog, D-Asp content is differently correlated with gonadal and plasmatic levels of testosterone, depending on the sex. In fact, the amount of the D-Asp is inversely linked with that of the testosterone in the ovary, while this correlation directly matched in the testis. In vivo short-term experiments, consisting of a single intra-peritoneal injection of D-Asp (2.0 mumol/g body weight), demonstrated that the enantiomer is significantly accumulated by both the ovary and testis, reaching after 3 h the highest uptake and thereafter decreasing to baseline values within 24 h. Furthermore, D-Asp influences the synthesis and/or the release of testosterone, causing a decrease of its level in the female, and an increase in the male, respectively. In vivo long-term experiments, D-Asp, chronically administered to the frogs of both sexes, enhances the maturation of both gonads, determining in the oocytes an higher accumulation of carbohydrate yolk plates in the ooplasm, and stimulating the spermatogenesis in the testis. Taken altogether, our results show that D-Asp operates differently in female and male frog gonads, indicating that it has different targets in the reproductive machinery depending on the sex.

There is evidence that certain phytoestrogens inhibit aromatase, the enzyme that converts androgens to oestrogens. Kinetic studies in cell-free preparations show that they may inhibit aromatase by competitive binding to the enzyme, but there is a paucity of studies investigating longer-term effects of phytoestrogens on the expression of steroidogenic enzymes. This study tested the hypothesis that phytoestrogens could reduce aromatase activity by down-regulation of its expression. Experiments were carried out on primary cultures of human granulosa-luteal (GL) cells after they had been exposed to phytoestrogens for 48 h. Aromatase activity was measured by the ability of cells to convert testosterone to estradiol over a 4h period and aromatase mRNA expression (mRNA(arom)) was subsequently measured from the same cells using quantitative real-time PCR. The compounds investigated were the flavones, apigenin and quercetin, and the isoflavones, genistein, biochanin A and daidzein at doses of 10 microM and 100 nM. Combinations of these compounds at the lower dose were also investigated. All compounds tested dose-dependently reduced mean mRNA(arom) compared with controls. Apigenin was the most potent inhibitor with significant inhibition of mRNA(arom) seen at both 10 microM and 100 nM, whilst other flavonoids (except biochanin A) only induced significant inhibition (p

Richter-Unruh, A., N. Jorch, et al. (2002). "Venous sampling can be crucial in identifying the testicular origin of idiopathic male luteinising hormone-independent sexual precocity." Eur J
It has been recently shown that male LH-independent sexual precocity is caused by a somatic activating mutation in the luteinising hormone receptor (LHR) of Leydig cell tumours. In each of the patients described to date, the tumour was a well-defined, single encapsulated nodule. We present a 5.7-year-old boy with nodular Leydig cell hyperplasia, who harbours a somatic mutation of the LHR gene. The boy showed the clinical features of severe sexual precocity caused by LH-independent testosterone hypersecretion. Congenital adrenal hyperplasia, hCG- or androgen-secreting tumours, McCune-Albright syndrome, and familial male-limited precocious puberty (or testotoxicosis) were all ruled out as possible causes. A hypoechoic area was detected at the cranial pole of his right testis and a biopsy was performed. Histological examination revealed a lack of mature Leydig cells. When DNA from the affected tissue was isolated and sequenced, no somatic mutation of the LHR gene was found. To further determine the origin of the elevated testosterone levels, venous sampling was performed. Blood samples taken from the right spermatic vein showed an elevated serum testosterone concentration of 259 nmol/l. Unilateral orchiectomy of the right testis was performed, and systemic testosterone concentrations normalised. Histological examination revealed nodular Leydig cell hyperplasia. DNA analysis of the nodular tissue showed a heterozygous mutation in exon 11 of the LHR gene, which caused the replacement of aspartic acid at codon 578 by histidine. CONCLUSION: the somatic activating mutation (Asp578His) of the luteinising hormone receptor gene is not only present in Leydig cell adenomas, but can also be found in nodular Leydig cell hyperplasia. Venous sampling can play a vital role in determining the origin of elevated testosterone levels.


We investigated a possible modulation of growth hormone (GH) secretion by testosterone by measuring the growth hormone releasing hormone (GHRH)-stimulated and N-methyl-d,l-aspartic acid (NMA)-induced GH secretion in adult rhesus monkeys. Intact, orchidectomized and testosterone-substituted (testosterone enanthate 125 mg/week, i.m. for 5 weeks) orchidectomized monkeys (n=5) were used in the study. GHRH (25 microg/kg body weight) or NMA (15 mg/kg body weight) was infused through a Teflon cannula implanted in the saphenous vein. Sequential blood samples were collected 30-60 min before and 60 min after the injection of the neurohormone or the drug at 10-20-min intervals. All bleedings were carried out under ketamine hydrochloride anaesthesia (initial dose 5 mg/kg body weight i.m., followed by 2.5 mg/kg at 30-min intervals). The plasma concentrations of GH, testosterone and oestradiol (E(2)) were determined by using specific assay systems. Administration of GHRH elicited a significant increase in GH secretion in all three groups of animals. There was no significant difference in the responsiveness of pituitary somatotrophs to exogenous GHRH challenges between intact and orchidectomized monkeys and testosterone replacement in orchidectomized animals did not significantly alter the GHRH-induced GH response. The responsiveness of hypothalamic GHRH neurones apparently did undergo a qualitative change after orchidectomy, as GH response to NMA was less in orchidectomized animals than in intact monkeys. The responsiveness of GHRH neurones to exogenous NMA was restored and even potentiated when orchidectomized monkeys were treated with testosterone. Taken together, these findings suggest that testosterone does not affect the sensitivity of the pituitary somatotrophs to GHRH but stimulates the secretion of GH by modulation of the NMDA drive to GHRH neurones.

The hydrolysis product of glucobrassicin, indole-3-carbinol (I3C), is metabolized to a variety of products, including the dimeric 3,3′-diindolylmethane (DIM). Both I3C and DIM exert a variety of biological and biochemical effects. Most of these effects appear to occur because I3C modulates several nuclear transcription factors. I3C induces phase I and phase II enzymes that metabolize carcinogens, including estrogens. Administration of either I3C or DIM results in increased 2-hydroxylation of estrogens. I3C also enhances DNA repair by affecting several of the proteins involved in this process. I3C induces both G1 cell cycle arrest and apoptosis. All of these activities lead to anticancer effects. Although I3C has been shown to protect against tumor induction by some carcinogens, it has also been observed to promote tumor development in animal models. In humans, I3C and DIM affect the metabolism of estrogens. Concerns have been raised that I3C might increase the formation of estrogen metabolites that induce or promote cancer, but this has not been demonstrated. I3C has been found to be effective in treating some cases of recurrent respiratory papillomatosis, and it may have other clinical uses.


A cDNA, corresponding to a rat submandibular mRNA which is accumulated at a 20-fold higher level in males than females, has been isolated. The predicted protein, SMR2, has a calculated molecular mass of 15.4 kDa and is rich in glutamine/glutamic acid, proline, and asparagine/aspartic acid, a characteristic of the so-called salivary glutamine-rich proteins (GRPs) of the submandibular gland of rats. Nucleotide sequence comparisons indeed revealed strong similarities between the sequences of the SMR2 mRNA and that of GRPs, except in the region encoding the carboxyl-terminal part of the proteins. In particular, the SMR2 mRNA contains the 5′-untranslated region and the signal peptide region shared by both groups of GRPs and proline-rich proteins (PRPs). A major difference is that, in SMR2, the peptidic motif which is repeated four or five times in GRPs, is only found once. The SMR2 gene is about 3.5 kilobases in length and contains 4 exons. The second intron, which does not exist in characterized GRP genes, splits the "transition" region which separates the repetitive sequences from the signal peptide. This structure is reminiscent of that found in most PRP genes, strengthening the hypothesis that GRP and PRP genes have the same ancestral origin.


Here we present a new family of endogenous peptides identified in rat testis with structure of glutamyl-tripeptide amides which are also present in plasma. These peptides have different activities in the hypophyseal-gonadal axis. Evidences showing the endocrine activities of some of the peptides are presented. In this communication we demonstrate the presence of peptides with a common structure
Glu-X-Pro amide, where X can be one of the following amino acids: glutamic acid, glutamine, aspartic acid, asparagine, phenylalanine or tyrosine. These peptides have been identified by a series of chromatographies and by mass spectrometry. Some of the peptides where tested for its biological activity observing that subcutaneous administration of the peptides Glu-Glu-Pro amide, Glu-Gln-Pro amide and Glu-Phe-Pro amide were able to reduce plasma levels of testosterone and luteinizing hormone (LH) without modification of the levels of follicle stimulating hormone (FSH). The peptide Glu-Asp-Pro amide, however, produced an increase in the levels of testosterone without modifying LH or FSH levels. It is proposed that the glutamyl-tripeptide amides that reduce the levels of testosterone and LH are released from the testis and act in the pituitary via circulation in an endocrine manner. The specific inhibition of LH release is similar to that produced by inhibit on FSH release. On the other hand the peptide that increases the levels of testosterone is produced in the testis and seems to act directly in the testis in a paracrine or autocrine manner. It is proposed here a new mechanism of regulation of hypophyseal-gonadal axis, a negative feedback exerted by the glutamyl-tripeptide amides in the pituitary. Also it is proposed the generic name of gonadins for the novel family of glutamyl-tripeptide amides. We suggest that gonadins could be used in the future as drugs for treatment of different endocrine disorders, hormone-dependent cancer and as contraceptives.


In the current study, localization of D-aspartic acid (D-Asp) in rat testis was studied by immunohistochemical and biochemical techniques. Immunohistochemical staining of this tissue using specific polyclonal antibody to D-Asp revealed D-Asp immunoreactivity (IR) in the cytoplasm of germ cells, especially around the region rich in elongate spermatids, the most mature of the germ cells. Weak IR was also noted in cytoplasm of spermatocytes and round spermatids; however, it was negligible in interstitial cells and Sertoli cells. The intensity of immunostaining in each seminiferous tubule differed according to its distinct germ cell composition. In testis of young rats, seminiferous tubules lack elongated spermatids, and D-Asp was found to be localized in spermatocytes, the most mature population of germ cells at that age. We used various toxicants to destroy specific testicular cell populations and to confirm the localization of D-Asp in rat testis. Administration of ethane dimethane sulfonate induced a selective destruction of all Leydig cells in this tissue. This resulted in a significant decrease in the D-Asp level, which was probably due to a drop in testosterone brought about by this treatment, and this was followed by a modulation of spermatogenesis. Three days after treatment with methoxyacetic acid (MAA), many seminiferous tubules were found to lack or to have severe depletions of pachytene spermatocytes, but not of elongate spermatids. This caused reductions in protein content and in the total amount of L-Asp, but not that of D-Asp. Twenty days after treatment with MAA, the depleted population of germ cells progressed through the spermatogenic cycle from pachytene spermatocytes to elongate spermatids. At this time, the level of D-Asp decreased significantly, as did that of L-Asp and protein, consistent with D-Asp localization in elongate spermatids. This decrease in the D-Asp level was also seen with immunostaining.

Flavonoids and related structures (e.g., flavones, isoflavones, flavanones, catechins) exert various biological effects, including anticarcinogenic, antioxidant and (anti-)estrogenic effects, and modulation of sex hormone homeostasis. A key enzyme in the synthesis of estrogens from androgens is aromatase (cytochrome P450 19; CYP19). We investigated the effects of various natural and synthetic flavonoids on the catalytic activity and promoter-specific expression of aromatase in H295R human adrenocortical carcinoma cells. Natural flavones were consistently more potent inhibitors than flavanones. IC(50) values for 7-hydroxyflavone, chrysin, and apigenin were 4, 7, and 20 microM, respectively; for the flavanones 7-hydroxyflavanone and naringenin the IC(50) values were 65 and 85 microM, respectively. The steroidal aromatase inhibitor (positive control) 4-hydroxyandrostenedione had an IC(50) of 20 nM. The inhibition by apigenin and naringenin coincided with some degree of cytotoxicity at 100 microM. The natural flavonoid derivative rotenone (IC(50) 0.3 microM) was the most potent aromatase inhibitor tested. Several synthetic flavonoid and structurally related quinolin-4-one analogs inhibited aromatase activity. The most potent inhibitor was 4’-tert-butyl-quinolin-4-one (IC(50) 2 microM), followed by two 2-pyridinyl-substituted alpha-naphthoflavones (IC(50)s 5 and >30 microM). The two 2-pyridinyl-substituted gamma-naphthoflavones consistently produced biphasic concentration-response curves, causing about 1.5-fold aromatase induction at concentrations below 1 microM and inhibition above that level (IC(50)s 7 and >30 microM). The natural flavone quercetin and isoflavone genistein induced aromatase activity 4- and 2.5-fold induction, respectively, at 10 microM. This coincided with increased intracellular cAMP concentrations and increased levels of the cAMP-dependent pII and to a lesser extent 1.3 promoter-specific aromatase transcripts. These results shed light on the structure-activity relationships for aromatase inhibition as well as mechanisms of induction in human H295R cells.


Rat testicular cells in culture produce several metalloproteinases including type IV collagenases (Sang et al. Biol Reprod 1990; 43:946-955, 956-964). We have now investigated the regulation of testicular cell type IV collagenase and other metalloproteinases in vitro. Soluble laminin stimulated Sertoli cell type IV collagenase mRNA levels. However, three peptides corresponding to different domains of the laminin molecule (CSRAKQAASIKVASADR, FALRGDNP, CLQDGDVRV) did not influence type IV collagenase mRNA levels. Zymographic analysis of medium collected from these cultures revealed that neither soluble laminin nor any of the peptides influenced 72-kDa type IV collagenase protein levels. However, peptide FALRGDNP resulted in both, a selective increase in two higher molecular-weight metalloproteinases (83 kDa and 110 kDa and in an activation of the 72-kDa rat type IV collagenase. Interleukin-1, phorbol ester, testosterone, and FSH did not affect collagenase activation. Immunocytochemical studies demonstrated that the addition of soluble laminin resulted in a redistribution of type IV collagenase from intracellular vesicles to the cell-substrate region beneath the cells. Peptide FALRGDNP induced a change from a vesicular to peripheral plasma membrane type of staining pattern. Zymography of plasma membrane preparations demonstrated triton-soluble gelatinases of 76 kDa, 83 kDa, and 110 kDa and a triton-insoluble gelatinase of 225 kDa. These results indicate that testicular cell type IV collagenase mRNA levels, enzyme activation, and distribution are influenced by laminin and RGD-containing peptides.

While a germline activating mutation of the luteinizing hormone receptor (LHR) gene is known to cause autonomous production of testosterone from testicular Leydig cells in male-limited precocious puberty, only a few studies have addressed the role of somatic LHR mutation in testicular pathology. The authors report a case of a 6-year-old boy who developed secondary sex characteristics including facial acne, enlarging genitalia, and aggressive behavior, for which serial biochemical evaluation confirmed the status of peripheral precocious puberty. Examination revealed asymmetrical testicular volume, following which a left testicular tumor was detected through ultrasonography. A left orchiectomy was performed, and histopathology revealed a well-circumscribed Leydig cell tumor. Molecular study of the exon 11 of the LHR gene revealed a missense mutation at the nucleotide position 1,732, leading to a substitution of histidine for aspartic acid at codon 578. Interestingly, the substitution was consistent with all previously reported LHR alteration in pediatric Leydig cell adenoma, but which had never before been reported in male-limited precocious puberty, suggesting that the mutation is a molecular signature of the adenoma.


Epidemiological and dietary studies have revealed an association between high dietary intake of cruciferous vegetables and decreased prostate cancer risk. Our studies have shown that indole-3-carbinol (I3C), a common phytochemical in cruciferous vegetables, and its in vivo dimeric product 3,3'-diindolylmethane (DIM) upregulate the expression of phase I and phase II enzymes, suggesting increased capacity for detoxification and inhibition of carcinogens. Studies from our laboratory and others have found that I3C can induce G1 cell-cycle arrest and apoptosis in prostate cancer cells. In addition, we found, by microarray gene expression profiling, that I3C and DIM regulate many genes that are important for the control of cell cycle, cell proliferation, signal transduction, and other cellular processes, suggesting the pleiotropic effects of I3C and DIM on prostate cancer cells. We recently found that I3C functions as an inhibitor of Akt and nuclear factor kappaB (NF-kappaB), which play important roles in cell survival and which are believed to be potential targets in cancer therapy. Studies have already shown that the inactivation of Akt and NF-kappaB is responsible for chemosensitization of chemoresistant cancer cells. Because there is no effective treatment strategy for hormone-dependent and, most importantly, hormone-independent and metastatic prostate cancer, our strategies to sensitize prostate cancer cells to a chemotherapeutic agent by I3C and DIM is a novel breakthrough that could be used for devising novel therapies for prostate cancer. In conclusion, the results from our laboratory and from others provide ample evidence for the benefit of I3C and DIM for the prevention and the treatment of prostate cancer.


Cancer cells exhibit deregulation in multiple cellular signaling pathways. Therefore, treatments using specific agents that target only one pathway usually fail in cancer therapy. The combination treatments using chemotherapeutic agents with distinct molecular mechanisms are considered more promising for higher efficacy; however, using multiple agents contributes to added toxicity. Emerging evidence has
shown that some "natural products" such as isoflavones, indole-3-carbinol (I3C) and its in vivo dimeric product 3,3'-diindolylmethane (DIM), and curcumin among many others, have growth inhibitory and apoptosis inducing effects on human and animal cancer cells mediated by targeting multiple cellular signaling pathways in vitro without causing unwanted toxicity in normal cells. Therefore, these non-toxic "natural products" from natural resources could be useful in combination with conventional chemotherapeutic agents for the treatment of human malignancies with lower toxicity and higher efficacy. In fact, recently increasing evidence from pre-clinical in vivo studies and clinical trials have shown some success in support of the use of rational design of multi-targeted therapies for the treatment of cancers using conventional chemotherapeutic agents in combination with "natural products". These studies have provided promising results and further opened-up newer avenues for cancer therapy. In this review article, we have succinctly summarized the known effects of "natural products" especially by focusing on isoflavones, indole-3-carbinol (I3C) and its in vivo dimeric product 3,3'-diindolylmethane (DIM), and curcumin, and provided a comprehensive view on the molecular mechanisms underlying the principle of cancer therapy using combination of "natural products" with conventional therapeutics.


Cancer cells are known to have alterations in multiple cellular signaling pathways and because of the complexities in the communication between multiple signaling networks, the treatment and the cure for most human malignancies is still an open question. Perhaps, this is the reason why specific inhibitors that target only one pathway have been typically failed in cancer treatment. However, the in vitro and in vivo studies have demonstrated that some natural products such as isoflavones, indole-3-carbinol (I3C), 3,3'-diindolylmethane (DIM), curcumin, (-)-epigallocatechin-3-gallate (EGCG), resveratrol, lycopene, etc, have inhibitory effects on human and animal cancers through targeting multiple cellular signaling pathways and thus these "natural agents" could be classified as multi-targeted agents. This is also consistent with the epidemiological studies showing that the consumption of fruits, soybean and vegetables is associated with reduced risk of several types of cancers. By regulating multiple important cellular signaling pathways including NF-kappaB, Akt, MAPK, Wnt, Notch, p53, AR, ER, etc, these natural products are known to activate cell death signals and induce apoptosis in pre-cancerous or cancer cells without affecting normal cells. Therefore, non-toxic "natural agents" harvested from the bounties of nature could be useful either alone or in combination with conventional therapeutics for the prevention of tumor progression and/or treatment of human malignancies.


PURPOSE: We investigated the effects of long-term testosterone replacement on copulatory behavior and dopaminergic neurotransmission in the medial preoptic area of aged male rats. MATERIALS AND METHODS: The rats were divided into 3 groups depending on testosterone replacement. Those in the long-term replacement group were castrated at the age of 12 months and received testosterone replacement thereafter for 12 months. In the short-term replacement group, rats were castrated at the age of 22 months and high or low dose testosterone replacement was done for 2 months. The control group consisted of aged rats 24 months old and young rats 12 weeks old, neither of which had been
castrated or received testosterone replacement. We observed sexual behavior in rats of these groups. After a behavioral test, we measured the tissue concentration of dopamine in the MPOA and the change rate of the extracellular dopamine level induced by infusion of N-methyl-D-aspartic acid (NMDA) in the MPOA and compared the long-term replacement and no-replacement groups. RESULTS: The rats in the long-term replacement group showed a mount rate at the same level as that of young rats at 6 weeks after starting replacement and it was maintained to 24 months of age. Their mount rate was significantly higher than that of the rats with the short-term replacement. A significantly higher change rate of dopamine release was recognized in the long-term group; however, no significant difference in the concentration of dopamine was recognized between aged rats with long-term replacement and those without replacement. CONCLUSIONS: Aged rats (24 months old) with long-term testosterone replacement maintained almost the same level of mount behavior as young rats (12 weeks old). The results imply that long-term testosterone replacement may favorably alter the decline in the process of sexual activity with aging. The restoration by testosterone replacement of dopaminergic activity in the MPOA may be involved in the maintenance of sexual function in aged rats.


Studies performed with animals suggest neurosteroid involvement in neuroprotection. However in humans, the role of neurosteroidogenesis in the regulation of degenerative processes is unknown. To determine whether cellular factors intervening in degenerative mechanisms may interfere with the process of neurosteroidogenesis in humans, we combined pulse-chase experiments with HPLC and continuous flow scintillation detection to compare neurosteroid production in normal and transfected SH-SY5Y cells with key proteins involved in Alzheimer's disease (AD). Microscope analyses revealed that cell morphology was unchanged in stably transfected SH-SY5Y cells overexpressing human native tau (hTau40), mutant tau (P301L), and wild-type amyloid precursor protein (APPwt) compared to controls. Biochemical investigations showed that hTau40 enhanced progesterone (PROG), 17OHPROG, testosterone, and 3alpha-androstanediol neosynthesis from pregnenolone. In contrast, tau with the pathogenic P301L mutation was devoid of action on neurosteroidogenesis. Overexpression of APPwt inhibited PROG formation, did not affect 17OHPROG and testosterone, but increased 3alpha-androstanediol and estradiol synthesis. Extracellular treatment of control cells with aggregated amyloid peptide mimicked the action of APPwt expression on PROG but not on 3alpha-androstanediol and estradiol production. Moreover, PROG biosynthesis in APPwt cells was up-regulated in the presence of a gamma-secretase inhibitor. Our results provide the first evidence for the regulation of neurosteroid biosynthesis by key proteins involved in the etiology of AD. The data suggest that pathogenic factors may induce neurodegeneration in humans through the reduction of the synthesis of endogenous neuroprotective neurosteroids in nerve cells.


N-methyl-D,L-aspartic acid (NMA), a potent neuroexcitatory and neurotoxic glutamic acid analogue, acutely elevated serum luteinizing hormone (LH) in male rats when given subcutaneously in doses below those that cause morphologically detectable hypothalamic neurotoxicity. NMA treatment in doses known to be subtoxic by morphological criteria fails to induce any permanent neuroendocrine dysfunction as
assessed by several physiological parameters, including NMA responsiveness after multiple consecutive doses spaced at 24 h intervals, subsequent basal LH levels and subsequent postcastration LH elevations. Like naloxone, NMA elevates serum LH by reversibly stimulating a central labile pool. Neither has a direct stimulatory effect on the pituitary in vitro. Treatment with either attenuates naloxone-induced LH stimulation 2 h, but not 14 days, later while pituitary responsiveness to LHRH in vivo remains unaltered. Neither NMA nor naloxone is dependent upon testosterone for its LH stimulatory action and both increase serum LH through physiological mechanisms responsive to testosterone inhibition. It is concluded that subtoxic LH stimulating doses of NMA provide a useful tool in discerning neurotransmitter systems involved in central control of the hypothalamic-pituitary-gonadal axis.


The effects of aspartic acid (aa) on ventilation were evaluated in awake male and female rats prior to and 15, 30, and 45 minutes after saline, 100 mg/kg or 580 mg/kg aa was injected subcutaneously. Subsequently, rats were exposed to hypoxic and hypercapnic gas challenges. In males, 100 mg/kg aa increased ventilation (VE) by increasing inspiratory flow rate (VT/TI), tidal volume (VT), and frequency of breathing (f) by 30 minutes, whereas in females VT was increased above saline levels only at 15 minutes. VE did not decrease over time. A dose of 580 mg/kg aa depressed ventilation in males for 2 hours by decreasing VT, VT/TI and f. In contrast, female rats exhibited a decreased ventilation only at 15 minutes which then began to return to saline levels by 45 minutes. Neither male nor female rats treated with either dose of aa showed a depressed response to hypoxia or hypercapnia. These data indicate that aa at two doses can affect the pattern of ventilation differently in male and female rats. One mechanism responsible for the differences noted between the two groups is the effect aspartic acid may have on testosterone production. An additional study comparing ventilatory responses of sham operated and castrated males to various doses of aa indicated that testosterone was not necessary to show the 'male' pattern of response.


We have previously shown that subcutaneous administration of aspartic acid (a dicarboxylic acidic amino acid) at a dose of 580 mg/kg causes long lasting depression of ventilation in adult intact and postpubertally castrated male rats, but not in intact female rats. The purpose of the present study was to determine if hypogonadism induced by perinatal administration of testosterone propionate (TP) will alter ventilation, oxygen consumption, and the ventilatory response to aspartic acid and to hypercapnia in adult males. TP treatment resulted in adult males who had lower body, prostate, heart, and testes weights than those of control male rats. Ventilation in air and oxygen consumption were comparable between the two groups as was the ventilatory response to aspartic acid. In contrast, TP-treated rats exhibited a significantly decreased ventilatory response to hypercapnia due predominantly to lower tidal volumes compared to control animals. Aspartic acid treatment did not affect oxygen consumption in either group. Thus, TP treatment results in the development of adult male rats who, although hypogonadal, retain a male-like ventilatory response to aspartic acid, but whose response to hypercapnia is more like that of hypogonadal men and rats.

Previously we observed that acute subcutaneous administration of aspartic acid (580 mg/kg) depressed ventilation in awake male, but not female, rats, suggesting that this agent may be used as a marker for sexual dimorphism in the control of ventilation. Moreover, males castrated postpubertally showed a response similar to that of intact male rats. Thus the hormonal milieu of male rats appear not to be necessary to elicit the masculine type of ventilatory response to aspartic acid. The purpose of this study was 1) to determine whether adult female rats androgenized by the administration of testosterone propionate (TP) 1 day after birth would alter their ventilation in response to aspartic acid to be more malelike and 2) to compare these results with those of intact (I) and ovariectomized (O) female rats. Minute ventilation and O2 consumption in air and in response to aspartic acid administration were evaluated in awake animals in all three groups. Furthermore the minute ventilation of all rats to a hypercapnic challenge was also evaluated. Ovariectomy resulted in rats increased body weights but decreased weight-corrected ventilation and O2 consumption compared with TP-treated and I animals. Minute ventilation after hypercapnic challenge in the three groups was similar. TP-treated rats responded to aspartic acid administration with a marked depression of ventilation similar to that previously noted in males, whereas neither I nor O rats showed such a response. The depression of ventilation in the TP-treated group in response to aspartic acid was not a consequence of a depression of O2 consumption. (ABSTRACT TRUNCATED AT 250 WORDS)


This study was designed to establish whether 3,3'-diindolylmethane (DIM) can inhibit cervical lesions, alter estrogen metabolism in favor of C-2 hydroxylation, and enhance immune function in the K14-HPV16 transgenic mouse model. Mice were bred, genotyped, implanted with E(2) pellets (0.25 mg/90-day release) under anesthesia, and divided into groups. Wild-type and transgenic mice were given either AIN76A diet alone or with 2,000 ppm DIM for 12 weeks. Blood and reproductive tracts were obtained. Blood was analyzed for estrogen metabolites and IFN-gamma. The cervical transformation zone was sectioned and stained for histology. Estradiol C-2 hydroxylation and serum IFN-gamma levels were significantly increased over controls in wild-type and transgenic mice receiving DIM. In wild-type mice without DIM, hyperplasia of the squamous epithelium was observed. Wild-type mice fed DIM displayed a normal thin epithelium. In transgenic mice without DIM, epithelial cell projections into the stroma (papillae) were present. An additional degree of nuclear anaplasia in the stratum espinosum was observed. Dysplastic cells were present. Transgenic mice fed DIM displayed some mild hyperplasia of the squamous epithelium. DIM increases estrogen C-2 hydroxylation in this model. Serum INF-gamma was increased, indicating increased immune response in the DIM-fed animals. Histopathology showed a marked decrease in cervical dysplasia in both wild-type and transgenic mice, indicating that DIM delays or inhibits the progression from cervical dysplasia to cervical cancer. Using the K14-HPV16 transgenic mouse model, we have shown that DIM inhibits the development of E6/E7 oncogene-induced cervical lesions.

We hypothesized that administration of estradiol benzoate to males and testosterone propionate to female neonatal rat pups alters sex-specific ventilatory responses to aspartic acid with correspondent changes in N-methyl-D-aspartate receptor subunit 1 (NR1) expression determined by Western blot in specific brain regions. One-day-old rat pups received estradiol benzoate, testosterone propionate, or vehicle and were studied at weanling and adulthood. Different groups had distinct patterns of changes in tidal volume and frequency of breathing after aspartic acid administration. NR1 expression in hypothalamus was altered by age, sex, and treatment. Medullary and pontine NR1 expression correlated with baseline ventilation and magnitude of the ventilatory response to aspartic acid in some groups. Thus 1) tidal volume and breathing frequency patterns in response to aspartic acid are gender, age, and treatment dependent; 2) sex, age, and exogenous steroid hormones affect NR1 expression primarily in the hypothalamus; and 3) there is correlation between NR1 expression in pons and medulla with ventilatory parameters.


Prokineticin 2 (Prok2) or prokineticin-receptor2 (Prok-R2) gene mutations are associated with Kallmann syndrome (KS). We describe a new homozygous mutation of Prok-R2 gene in a man displaying KS with an apparent reversal of hypogonadism. The proband, offspring of consanguineous parents, presented at age 19 years with absent puberty, no sense of smell, low testosterone and gonadotrophin levels. Magnetic resonance imaging showed olfactory bulb absence. The patient achieved virilization and spermatogenesis with gonadotrophin administration. Two years after discontinuing hormonal therapy, he maintained moderate oligozoospermia and normal testosterone levels. Prok2 and Prok-R2 gene sequence analyses were performed. The proband had a homozygous mutation in Prok-R2 exon 2 that harbours the c.T820>A base substitution, causing the introduction of an aspartic acid in place of valine at position 274 (Val274Asp). His mother had the same mutation in heterozygous state. This report describes a novel homozygous mutation of Prok-R2 gene in a man with variant KS, underlying the role of Prok-R2 gene in the olfactory and reproductive system development in humans. Present findings indicate that markedly delayed activation of gonadotrophin secretion may occur in some KS cases with definite gene defects, and that oligozoospermia might result from a variant form of reversible hypogonadotrophic hypogonadism.

We have previously observed that the quadruple (S407T-N417D-A419T-K473M) and triple (S407T-N17D-A419T) mutants of the chimeric construct of P450 2B1/2B2 do not undergo mechanism-based inactivation by 17alpha-ethynylestradiol (17EE) and tert-butyl 1-methyl-2-propynyl ether (tBMP). The ability of these mutants to metabolize 17EE, benzphetamine, and testosterone has been investigated. The profile for 17EE metabolism by both mutants was characteristic of both wild-types. The two mutants metabolized testosterone to form androstenedione with no formation of the hydroxy products as was seen with both the wild-types. Benzphetamine metabolism by the mutants showed that both mutants exhibited an increased tendency to catalyze demethylation rather than debenzylation. In the presence of the alternate oxidants cumene hydroperoxide and tert-butyl hydroperoxide, the wild-type 2B1 was not inactivated by 17EE. Metabolism of 17EE by 2B1 supported by these alternate oxidants revealed differences in the metabolites that may be related to the inability of 2B1 to be inactivated under these conditions.


In a randomly allocated, double-blind, placebo-controlled, crossover design, 50 patients with mild to moderate erectile dysfunction (ED) were treated for 1 month with placebo or a combination of L-arginine aspartate and Pycnogenol (Prelox). Patients reported sexual function from diaries. Testosterone levels and endothelial NO synthase (e-NOS) were monitored along with routine clinical chemistry. Intake of Pycnogenol for 1 month restored erectile function to normal. Intercourse frequency doubled. e-NOS in spermatozoa and testosterone levels in blood increased significantly. Cholesterol levels and blood pressure were lowered. No unwanted effects were reported. Prelox is a promising alternative to treat mild to moderate ED.


Androgen insensitivity syndrome (AIS) is an X-linked recessive disorder. The molecular mechanism of AIS is reduction or absence of androgen signalling caused by androgen receptor (AR) malfunction or absence. The phenotype of AIS varies from a complete female phenotype (complete AIS, CAIS) to male genitalia with mild hypospadias (partial AIS, PAIS). In the current study, we characterize a novel point mutation in the ligand binding domain of the AR gene in a 50-year-old Japanese CAIS patient. Sequence analysis showed a single point mutation at nucleotide 3359 (Genbank, NM 000044), T to C, in exon E in the AR gene. This mutation led to the conversion of codon 739 tyrosine into aspartic acid in the ligand binding domain. No specific androgen binding was detected in genital fibroblasts isolated from the patient. Transcriptional activating activity of the mutant AR was examined by transient DNA transfection into COS-1 cells. Wild-type AR successfully activated androgen inducible MMTV promoter dose-dependently. In contrast, the mutant AR did not activate MMTV promoter. Thus, we demonstrated the molecular characteristics of the novel point mutation in the ligand binding domain of the AR gene associated with
Saw palmetto extract (SPE), an extract from the ripe berries of the American dwarf palm, has been widely used as a therapeutic remedy for urinary dysfunction due to benign prostatic hyperplasia (BPH) in Europe. Numerous mechanisms of action have been proposed for SPE, including the inhibition of 5alpha-reductase. Today, alpha(1)-adrenoceptor antagonists and muscarinic cholinceptor antagonists are commonly used in the treatment of men with voiding symptoms secondary to BPH. The improvement of voiding symptoms in patients taking SPE may arise from its binding to pharmacologically relevant receptors in the lower urinary tract, such as alpha(1)-adrenoceptors, muscarinic cholinceptors, 1,4-dihydropyridine receptors and vanilloid receptors. Furthermore, oral administration of SPE has been shown to attenuate the up-regulation of alpha(1)-adrenoceptors in the rat prostate induced by testosterone. Thus, SPE at clinically relevant doses may exert a direct effect on the pharmacological receptors in the lower urinary tract, thereby improving urinary dysfunction in patients with BPH and an overactive bladder. SPE does not have interactions with co-administered drugs or serious adverse events in blood biochemical parameters, suggestive of its relative safety, even with long-term intake. Clinical trials (placebo-controlled and active-controlled trials) of SPE conducted in men with BPH were also reviewed. This review should contribute to the understanding of the pharmacological effects of SPE in the treatment of patients with BPH and associated lower urinary tract symptoms (LUTS).

We previously have shown that experimental diabetes in rats causes prostatic involution, reduces serum testosterone levels, and causes an upregulation in prostatic endothelin (ET) receptors. Furthermore, insulin treatment normalizes these changes (Saito et al., Mol Cell Biochem 210:1-12, 2000). Since experimental diabetes-induced reduction in serum testosterone may be a factor in the alteration of the ET receptors and of prostatic growth, we investigated the effect of castration, another means of involuting the prostate and decreasing serum testosterone levels, on the expression of ET receptors in ventral and dorsolateral regions of the rat prostate. Three-month-old Sprague-Dawley rats were surgically castrated or sham operated, and then killed on the 7th post-operative day. Biochemical and pharmacological properties, and localization of ET receptors in the rat prostate, were determined by performing a series of binding experiments with [(125)I]ET-1 and by light microscopy autoradiography, respectively. The expression levels of ET-1, ET-3, ET receptor subtypes and endothelin converting enzyme-1 (ECE-1) mRNAs were assessed by relative multiplex reverse transcription polymerase chain reaction (RT-PCR). The total density of ET receptors increases 3.7-fold in the ventral and 2.1-fold in the dorsolateral regions of the castrated rat prostate compared to sham operated animals. Castration causes a 2.4-fold increase in the density of alpha(1)-adrenoceptors (alpha(1)-ARs) in the ventral region of the prostate, but no change in the density of alpha(1)-ARs in the dorsolateral region of the rat prostate. The predominant ET receptor subtype in the rat prostate is the ETA subtype, which is mainly located in the prostatic stroma. In addition, RT-PCR data show an upregulation in the expression of ETB receptor subtype, ET-1 and ECE-1 mRNA in both regions, and a downregulation in the expression of ETA receptor subtype mRNA in the
The dorsolateral region of the castrated rat prostate. There is no change in the expression of ET-3 mRNA in either region. Castration does not cause significant changes in the pharmacological properties of prostatic ET receptors, i.e., the predominance of ETA receptors in either region of the prostate, or the expression of ETA receptor subtype mRNA in the ventral region of the castrated rat prostate. These results suggest the existence of a region/lobe-specific regulatory role for testosterone in the expression of the ET receptor system in the rat prostate.


OBJECTIVE: To investigate the effects of castration on the expression of endothelins (ETs), ET receptors and ET converting enzyme-1 (ECE-1) in the rat seminal vesicle (RSV). MATERIALS AND METHODS: Sprague-Dawley rats (3 months old) were surgically castrated or sham-operated, and then killed 7 days after surgery. Biochemical and pharmacological properties and the location of ET receptors in the RSV were determined by a series of binding experiments with [125I]ET-1, using membrane particulates and slide-mounted frozen sections of RSV. Expression levels of ETA and ETB receptor subtypes, ET-1, ET-3 and ECE-1 mRNAs were assessed by relative multiplex reverse-transcription polymerase chain reaction (RT-PCR). RESULTS: The density of total ET receptors increased significantly in the seminal vesicle of the castrated rat. The predominance of the ETA receptor subtype in the RSV did not change with castration. Autoradiographic studies showed the presence of ET receptors on the smooth muscle and epithelium of the RSV. In addition, RT-PCR showed an up-regulation in the expression of ETA and ETB receptor subtypes, ET-1 and ECE-1 mRNAs in the seminal vesicle of the castrated rat. However, castration caused no significant change in the expression levels of ET-3 mRNA. CONCLUSION: These findings suggest a regulatory role for testosterone in the expression of the ET receptor system in the RSV.


BACKGROUND: D-aspartic acid is an amino acid present in neuroendocrine tissues of invertebrates and vertebrates, including rats and humans. Here we investigated the effect of this amino acid on the release of LH and testosterone in the serum of humans and rats. Furthermore, we investigated the role of D-aspartate in the synthesis of LH and testosterone in the pituitary and testes of rats, and the molecular mechanisms by which this amino acid triggers its action. METHODS: For humans: A group of 23 men were given a daily dose of D-aspartate (DADAVIT) for 12 days, whereas another group of 20 men were given a placebo. For rats: A group of 10 rats drank a solution of either 20 mM D-aspartate or a placebo for 12 days. Then LH and testosterone accumulation was determined in the serum and D-aspartate accumulation in tissues. The effects of D-aspartate on the synthesis of LH and testosterone were gauged on isolated rat pituitary and Leydig cells. Tissues were incubated with D-aspartate, and then the
concentration (synthesis) of LH and cGMP in the pituitary and of testosterone and cAMP in the Leydig cells was determined. RESULTS: In humans and rats, sodium D-aspartate induces an enhancement of LH and testosterone release. In the rat pituitary, sodium D-aspartate increases the release and synthesis of LH through the involvement of cGMP as a second messenger, whereas in rat testis Leydig cells, it increases the synthesis and release of testosterone and cAMP is implicated as second messenger. In the pituitary and in testes D-Asp is synthesized by a D-aspartate racemase which convert L-Asp into D-Asp. The pituitary and testes possesses a high capacity to trapping circulating D-Asp from hexogen or endogen sources. CONCLUSION: D-aspartic acid is a physiological amino acid occurring principally in the pituitary gland and testes and has a role in the regulation of the release and synthesis of LH and testosterone in humans and rats.


The Center for Neurodegenerative Disease Research (CNDR) organized a 1 day symposium entitled "Emerging Alzheimer's disease Therapies: Focusing On The Future" on November 7th, 2001 at the University of Pennsylvania in Philadelphia, PA. The agenda (Fig. 1) focused on novel therapies for Alzheimer's disease (AD) designed to prevent/eliminate Abeta deposits in the brains of AD patients. While fibrillar Abeta deposits known as senile plaques (SPs) and intraneuronal tau fibrils known as neurofibrillary tangles (NFTs) are diagnostic of AD, >50% of patients with familial or sporadic AD as well as elderly Down's syndrome patients with AD harbor a third type of brain amyloid known as Lewy bodies formed by intraneuronal alpha-synuclein fibrils. Thus, AD is a "triple brain amyloidosis" since three different proteins (tau, alpha-synuclein) or peptide fragments (Abeta) of a larger Abeta precursor protein (APP) fibrillize and aggregate into pathological deposits of amyloid within (NFTs, LBs) and outside (SPs) neurons in AD brains. The symposium is summarized here followed by reviews from symposium speakers who describe potential anti-Abeta therapies some of which are in clinical trials.


Alopecia is a psychologically distressing phenomenon. Androgenetic alopecia (AGA) is the most common form of alopecia, which affects millions of men and women worldwide, and is an androgen driven disorder. To study the effect of beta-sitosterol phyto-vesicles on AGA, the testosterone-induced alopecia model was used. For the study, the albino rats were used and the period of study was 21 days. beta-Sitosterol is a phytosterol which is chemically similar to cholesterol. This compound was found suitable for the preparation of phyto-vesicles by the process involving its complexation with phosphatidyl choline. Pharmacokinetic studies of beta-sitosterol reveal its poor absorption through the intestine. The objective of the present study is to enhance the bioavailability of beta-sitosterol by its complexation with phosphatidyl choline. The complex of beta-sitosterol was prepared with phosphatidyl choline and then to formulate it as phyto-vesicles for the treatment of alopecia. The complex of beta-sitosterol was prepared with phosphatidyl choline and characterized on the basis of solubility, melting point, TLC, UV, IR and NMR spectroscopy. This complex was then formulated as phyto-vesicles and then characterized. The results revealed that effect on alopecia is better in case of phyto-vesicles as compared to the complex, physical mixture and the beta-sitosterol itself. Enhanced bioavailability of the beta-sitosterol complex may be due to the amphiphilic nature of the complex, which greatly enhance the water and lipid solubility of the compound. The present study clearly indicates the
superiority of phyto-vesicles over the complex and beta-sitosterol, in terms of better absorption and improved activity for the treatment of alopecia.


In the public opinion, phytochemicals (PCs) present in the human diet are often considered beneficial (e.g. by preventing breast cancer). Two possible mechanisms that could modulate tumor growth are via interaction with the estrogen receptor (ER) and inhibition of aromatase (CYP19). Multiple in vitro studies confirmed that these compounds act estrogenic, thus potentially induce tumor growth, as well as aromatase inhibitory, thus potentially reduce tumor growth. It is thought that in the in vivo situation breast epithelial (tumor) cells communicate with surrounding connective tissue by means of cytokines, prostaglandins and estradiol forming a complex feedback mechanism. Recently our laboratory developed an in vitro co-culture model of healthy mammary fibroblasts and MCF-7 cells that (at least partly) simulated this feedback mechanism (M. Heneweer et al., TAAP vol. 202(1): 50-58, 2005). In the present study biochanin A, chrysin, naringenin, apigenin, genistein and quercetin were studied for their estrogenic properties (cell proliferation, pS2 mRNA) and aromatase inhibition in MCF-7 breast tumor cells, healthy mammary fibroblasts and their co-culture. The proliferative potency of these compounds in the MCF-7 cells derived from their EC(50)s decreased in the following order: estadiol (4*10(-3) nM)>biochanin A (9 nM)>genistein (32 nM)>testosterone (46 nM)>naringenin (287 nM)>apigenin (440 nM)>chrysin (4 microM). The potency to inhibit aromatase derived from their IC(50)s decreased in the following order: chrysin (1.5 microM)>naringenin (2.2 microM)>genistein (3.6 microM)>apigenin (4.1 microM)>biochanin A (25 microM)>quercetin (30 microM). The results of these studies show that these PCs can induce cell proliferation or inhibit aromatase in the same concentration range (1-10 microM). Results from co-cultures did not elucidate the dominant effect of these compounds. MCF-7 cell proliferation occurs at concentrations that are not uncommon in blood of individuals using food supplements. Results also indicate that estrogenicity of these PCs is quantitatively more sensitive than aromatase inhibition. It is suggested that perhaps a more cautionary approach should be taken for these PCs before taken as food supplements.


Mechanisms regulating the expression of brain-derived neurotrophic factor, a member of the neurotrophin family, have been extensively studied in the rat cerebral cortex, hippocampus and cerebellum. In contrast, little is known regarding the regulation of this growth factor in the hypothalamus. Here we present an analysis of the regulation of brain-derived neurotrophic factor messenger RNA levels in chick embryo hypothalamic slice cultures following exposure to potassium chloride, glutamate agonists
and sex steroids. Following a week in chemically-defined media the tissue was depolarized by exposure to 50 mM potassium chloride for 6h, resulting in a significant 4.2-fold increase in the level of brain-derived neurotrophic factor messenger RNA. This result is consistent with studies of other brain regions. Similar 6-h acute exposures of the hypothalamic cultures to 25 microM N-methyl-D-aspartic acid, 25 microM kainic acid and 25 microM alpha-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid also significantly increased messenger RNA levels 2.5-, 2.1- and 1.4-fold, respectively. It was previously reported that brain-derived neurotrophic factor levels within the rat cerebral cortex, olfactory bulb and hippocampus are altered by exposure to 17beta-estradiol. Here we show that in hypothalamic slice cultures neither acute nor chronic treatments with 10 and 100 nM 17beta-estradiol and 10nM testosterone significantly altered the steady-state level of this growth factor. These findings show that neuronal activity, induced by glutamate agonists and potassium chloride, can regulate brain-derived neurotrophic factor messenger RNA levels within embryonic hypothalamic slice cultures. This regulation could play a critical role in the modulation of programmed cell death and synaptic maturation during development of the hypothalamus.


BACKGROUND AND OBJECTIVE: Mutations of the steroid 5alpha-reductase type 2 (SRD5A2) gene in karyotypic males result in a spectrum of external genitalia phenotypes ranging from complete female to nearly complete male. Here we performed genomic DNA analyses from individuals bearing the enzyme deficiency in order to detect the molecular abnormalities. PATIENTS: Four unrelated 46,XY patients of Mexican origin with ambiguous external genitalia were studied. A fertile, phenotypically normal male was also included. MEASUREMENTS: Coding sequence abnormalities of the SRD5A2 gene were assessed by exon-specific polymerase chain reaction, single-stranded conformational polymorphism and sequencing analysis. RESULTS: Five different missense mutations (two of them novel mutations) were identified. Three subjects presented homozygous single base mutations. These were located at exon 2 (G115D), exon 4 (P212R) and exon 5 (R246Q), and such changes have been described previously. The fourth patient was a compound heterozygote who presented two mutations located in exons 1 and 2. We found a hitherto unreported G --> A transition at the second nucleotide of codon 85 in exon 1 (GGC --> GAC), substituting glycine for aspartic acid (G85D). This patient also presented an identical alteration at codon 115 of exon 2, which was carried by his father (G115D). Finally, in another subject who was included originally as a control, we found a C --> A transversion (yet undescribed) at codon 245 in exon 5 (S245Y). CONCLUSIONS: Four different single base mutations that cause amino acid substitutions were detected in the steroid 5alpha-reductase type 2 gene of affected individuals. One patient and a normal control had two previously undescribed mutations. Although in the latter individual we cannot exclude the possibility that the base change is a genetic polymorphism, the molecular screening of 100 chromosomes suggests strongly that the change at codon 245 does represent a heterozygous mutation. Further studies, including the recreation of the mutations, will help to reveal the biochemical consequences resulting from these changes.


**3,3'-Diindolylmethane (DIM) is a potential chemopreventive phytochemical derived from Brassica**
vegetables. In this study we characterized the effect of DIM on cell cycle regulation in both androgen-dependent LNCaP and androgen receptor negative p53 mutant DU145 human prostate cancer cells. DIM had an anti-proliferative effect on both LNCaP and DU145 cells, as it significantly inhibited [3H]-thymidine incorporation. FACS analysis revealed a DIM-mediated G(1) cell cycle arrest. DIM strongly inhibited the expression of cdk2 and cdk4 protein and increased the expression of the cell cycle inhibitor p27(Kip1) protein in LNCaP and DU145 cells. Promoter deletion studies with p27(Kip1) reporter gene constructs showed that this DIM-mediated increase in p27(Kip1) was dependent on the Sp1 transcription factor. Moreover, using a dominant negative inhibitor of p38 MAPK, we showed that the induction of p27(Kip1) and subsequent G(1) arrest by DIM involve activation of the p38 MAPK pathway in the DU145 cells. Taken together, our results indicate that DIM is able to stop the cell cycle progression of human prostate cancer cells regardless of their androgen-dependence and p53 status, by differentially modulating cell cycle regulatory pathways. The Sp1 and p38 MAPK pathways mediate the DIM cell cycle regulatory effect in DU145 cells.


Hirschsprung disease (distal intestinal aganglionosis, HSCR) is a multigenic disorder with incomplete penetrance, variable expressivity, and a strong male gender bias. Recent studies demonstrated that these genetic patterns arise because gene interactions determine whether enteric nervous system (ENS) precursors successfully proliferate and migrate into the distal bowel. We now demonstrate that male gender bias in the extent of distal intestinal aganglionosis occurs in mice with Ret dominant-negative mutations (RetDN) that mimic human HSCR. We hypothesized that male gender bias could result from reduced expression of a gene already known to be essential for ENS development. Using quantitative real-time polymerase chain reaction (PCR) we demonstrated reduced levels of endothelin converting enzyme-1 and endothelin-3 mRNA in the male mouse bowel at the time that ENS precursors migrate into the colon. Other HSCR-associated genes are expressed at comparable levels in male and female mice. Testosterone and Mullerian inhibiting substance had no deleterious effect on ENS precursor development, but adding EDN3 peptide to E11.5 male RetDN heterozygous mouse gut explants in organ culture significantly increased the rate of ENS precursor migration through the bowel.


A wealth of preclinical evidence supports the antitumorigenic properties of indole-3-carbinol (I3C), which is a major bioactive food component in cruciferous vegetables. However, the underlying molecular mechanism(s) accounting for these effects remain unresolved. In the present study, estrogen receptor alpha (ER-alpha) was identified as a potential molecular target for I3C. Treating MCF-7 cells with 100 microM I3C reduced ER-alpha mRNA expression by approximately 60% compared to controls. This reduction in ER-alpha transcript levels was confirmed using real-time polymerase chain reaction. The I3C dimer, 3,3'-diindolylmethane (DIM), was considerably more effective in depressing ER-alpha mRNA in MCF-7 cells than the monomeric unit. The suppressive effects of 5 microM DIM on ER-alpha mRNA was
comparable to that caused by 100 microM I3C. DIM is known to accumulate in the nucleus and is a preferred ligand for aryl hydrocarbon receptor (AhR) to I3C. The addition of other AhR ligands, alpha-naphthoflavone (alpha-NF, 10 microM) and luteolin (10 microM), to the culture media resulted in a similar suppression in ER-alpha mRNA levels to that caused by 5 microM DIM. Thus, it is likely that the binding of ligands to AhR inhibits nuclear ER-alpha transcript. The results from these experiments suggest that the antitumorigenic effects of I3C in MCF-7 human breast cancer cells may arise from its ability to reduce ER-alpha expression through the binding of its metabolite, DIM, to the nuclear AhR.


BACKGROUND: Studies using purified enzyme preparations, placental microsomes or cell lines have shown that certain phytoestrogens can inhibit the enzymes that convert androgens to estrogens, namely aromatase and 17beta-hydroxysteroid dehydrogenase (HSD) type 1 and type 5. The study aim was to investigate the effects of selected phytoestrogens on aromatase and 17beta-HSD type 1 activity in primary cultures of human granulosa-luteal (GL) cells. METHODS AND RESULTS: GL cells, cultured for 48 h in medium containing 5% fetal calf serum and for a further 24 h in serum-free medium with or without hFSH or hCG, were exposed to steroid substrates during the last 1-4 h of the experiment. The production of progesterone in the presence of pregnenolone or estradiol synthesis from androstenedione, estrone or testosterone showed dose- and time-dependent increases. Whilst hCG priming had no effect on progesterone production, FSH priming induced mean 68 and 56% increases in the production of estradiol from androstenedione (A-dione) and estrone respectively, but had no significant effect on the metabolism of testosterone to estradiol. None of the phytoestrogens investigated had any acute effects on enzyme activity. In contrast, when GL cells were exposed to the compounds for 24 h prior to exposure to steroid substrates for 4 h, 10 micro mol/l apigenin and zearalenone significantly inhibited aromatase activity, whilst biochanin A and quercetin had no effect. None of the phytoestrogens inhibited FSH-induced 17beta-HSD type 1 activity, and only quercetin significantly inhibited progesterone production. CONCLUSIONS: The inability of phytoestrogens to acutely inhibit steroidogenic enzymes in human GL cells (as has been shown in cell-free models) suggests that they are either rapidly metabolized to relatively inactive compounds or that the high enzyme activity in human GL cells masks any inhibitory effects of the compounds at the concentration tested.


The effects of three acid condensation products of indole-3-carbinol (I3C), i.e. 3,3’-diindolylmethane (DIM), 5,6,11,12,17,18-hexahydrocyclonona[1,2-b:4,5-b’:7,8-b’] triindole (CTI) and 2,3-bis[3-indolylmethyl]indole (BII), on cytochrome P450 and phase II enzymes were studied in primary cultures of rat and cynomolgus monkey liver cells. In rat hepatocytes all three indole derivatives dose-relatedly induced the ethoxyresorufin O-dealkylation (EROD) activity (to 24-fold) and 7 alpha-hydroxylation of testosterone (to 4-fold), whereas all three decreased the 16 alpha- and 2 alpha-testosterone hydroxylation (DIM to 60%, CTI and BII to a mere 5% of the control cells). Treatment of monkey
hepatocytes with DIM and BII enhanced the EROD activity to 6- and 9-fold, respectively. Furthermore, BII decreased the 6 beta-hydroxylation of testosterone (to 60% of the untreated cultures) in monkey cells. Phase II enzymes were also affected. In rat hepatocytes DIM, CTI and BII enhanced DT-diaphorase (DTD) (= NAD(P)H-quinone reductase) activity, and DIM and BII the glucuronidation of 1-naphthol. In monkey cells BII only enhanced DTD, and no changes were observed in the glucuronidation of 1-naphthol after treatment with either DIM or BII. The indole derivatives did not affect glutathione S-transferase activity and sulfation of 1-naphthol in either rat or monkey hepatocytes. These results identify two novel acid condensation products of I3C, CTI and BII, as potent compounds in affecting biotransformation in rat as well as in monkey hepatocytes.


Groups of male Wistar rats were fed semi-synthetic diets containing 0, 200 or 500 mg indole-3-carbinol (I3C)/kg for 2, 7, 14 or 28 days. After 2 days, P-450 activities were already induced, but the isoenzyme pattern induced was different in the liver and the small intestine. Hepatic P4501A1, P4501A2 and P4502B1 apoprotein levels were dose-relatedly enhanced, whereas in the small intestine induced levels of P4502B1 and P4501A1 were detected but P4501A2 was not induced. Pentoxy- and ethoxyresorufin dealkylation (PROD and EROD) were dose-relatedly enhanced in the liver (5- and 7-fold, respectively, in the higher dose group) as well as in the small intestine (8- and 13-fold, respectively, at 500 mg I3C/kg diet). Testosterone 16 alpha- and 16 beta-hydroxylation in the small intestine were enhanced (6-9-fold) from day 2 onwards, but in the liver these activities were only slightly enhanced from day 7 onwards. Thus, the major forms induced in the liver appear to be P4501A1, P4501A2, P4502B1 and, to a lesser extent, P4503A, whereas in the small intestine all of the effects that were found are associated with only one cytochrome P-450, P4502B1. After 2 days I3C (500 mg/kg) induced glutathione S-transferase in the liver (1.3-fold) and small intestine (1.5-fold). Hepatic glucuronyl transferase (GT1) was induced (about 1.6-fold) after 7, 14 and 28 days. DT-diaphorase was induced in the liver (2.7-fold) and small intestine (1.5-fold) after 14 days of exposure to 500 mg I3C/kg diet. Treatment of rat hepatocytes with indole-3-acetonitrile and 3,3'-diindolylmethane, but not I3C and indole-3-carboxaldehyde, enhanced EROD activity and halved testosterone 16 alpha- and 2 alpha-hydroxylation. All four indoles slightly induced glutathione S-transferase in cultured hepatocytes. Thus, the in vitro studies suggest that the in vivo effects of I3C have to be attributed to indole-condensation products, such as 3,3'-diindolylmethane, but not to I3C itself.


OBJECTIVE: To study the active components and their functionary mechanism of the extract of Brassica alba seeds, which inhibits experimental mice prostatic hyperplasia. METHOD: Prostatic hyperplasia of castrated male mice induced by testosterone propionate, the penetrability of capillary vessel of mice skin induced by histamine and the endermic flesh bud of rat induced by filter paper were used as experimental models. Sinalbin and beta-sitosterol separated from seeds of Brassica alba were used to test the activities. RESULT: Sinalbin and beta-sitosterol(16.0 mg.kg-1.d-1 and 8.0 mg.kg-1.d-1) could significantly inhibit mice prostatic hyperplasia induced by testosterone propionate and activity of serum acid phosphatase(P < 0.01 or P < 0.05), Sinalbin(16.0 mg.kg-1.d-1)could significantly inhibit the hyperplasia of endermic flesh bud in
rat induced by filter paper ($P < 0.05$), beta-sitosterol ($16.0$ mg.kg$^{-1}$.d$^{-1}$ and $8.0$ mg.kg$^{-1}$.d$^{-1}$) could significantly decrease the penetrability of capillary vessel of mice skin induced by histamine.

CONCLUSION: Sinalbin and beta-sitosterol have anti-androgen and anti-inflammation activities.


Phytochemical investigation of the whole plant of Lepisorus contortus (Christ) Ching led to the isolation of five new phenylethanoid glycosides (1-5), each containing a caffeoyl group, a new flavonoid glycoside (10), and 14 known compounds (6-9 and 11-15, syringic acid, vanillic acid, phloretic acid, diplopterol, and beta-sitosterol). This is the first report of phenylethanoid glycosides from the family Polypodiaceae. Compounds 1-15 were evaluated for their cancer chemopreventive potential based on their ability to inhibit tumor necrosis factor alpha (TNF-alpha)-induced NF-kappaB activity, nitric oxide (NO) production, and aromatase, quinone reductase 2 (QR-2), and COX-1/-2 activities. Quercetin-3-O-beta-d-glucoside (15) demonstrated inhibition against QR2 with an IC(50) value of 3.84 muM, which confirmed kaempferol/quercetin glycosides as the active compounds to inhibit QR2. The compound also demonstrated NF-kappaB activity with an IC(50) value of 33.6 muM. In addition, compounds 1, 2, 4, and 6 showed aromatase activity with IC(50) values of 30.7, 32.3, 26.8, and 35.3 muM, respectively.


Familial male-limited precocious puberty (FMPP) is an autosomal dominant disorder characterized by marked elevation of serum testosterone despite low levels of gonadotropin. Recently, a single point mutation in the LH/hCG receptor (LH/CGR) gene was found in FMPP families that constitutively activates the LH/CGR, causing Leydig cell activation and precocious puberty. Among the Japanese population, only four sporadic cases of male-limited precocious puberty have been reported. In the current study, we examined one of the four reported Japanese patients with sporadic male-limited precocious puberty and found the same mutation as that in the FMPP families. Genomic DNA was isolated, and the polymerase chain reaction (PCR) was performed to amplify a fragment of LH/CGR DNA encoding amino acid residues that include transmembrane helixes 5 and 6. Sequencing of the PCR products revealed a heterozygous adenosine-guanine transition at nucleotide 1733 in codon 578. The mutation encodes an aspartic acid578-glycine substitution in transmembrane helix 6. The mutant LH/CGR, created by site-directed mutagenesis in vitro, exhibited constitutively higher cAMP levels in transfected COS-7 cells than the wild-type LH/CGR, as described previously; however, basal inositol phosphate levels were not increased by transfection with complementary DNA for the mutant receptor. The concentration and affinity of [125I]hCG-binding sites were similar in cells transfected with the mutant and wild-type LH/CGR complementary DNAs, indicating that the mutant did not alter the production of receptor or its ability to bind human LH/CG. The sporadic occurrence of this case was confirmed by further studies. The mutation creates a recognition site for the restriction endonuclease Mspl. Restriction digestion was positive for the mutant not digested by Mspl, indicating that the patient’s mutant allele was not inherited from his parents. DNA analysis of the patient and the parents, using microsatellite repeat markers, was compatible with biological paternity and maternity. We conclude that the aspartic acid578-->glycine mutation in the LH/CGR has arisen in the Japanese population and is the cause of a sporadic case of male-limited precocious puberty.
A single point mutation that encodes an aspartic acid (Asp578) to glycine substitution in the LH/CG receptor (LH/CGR) gene, D578G, was recently found in American patients with familial male-limited precocious puberty and in a Japanese patient with a sporadic form of the disorder. Transfection of the mutant, compared to the wild-type, LH/CGR complementary DNA into COS-7 cells results in higher basal cAMP production, but a normal agonist-induced response; the mutation is, therefore, proposed to constitutively activate Leydig cells and elevate serum testosterone, despite low levels of gonadotropin. In the current study we examined two additional Japanese patients with male-limited precocious puberty without a family history of the disease. We describe a heterozygous cytosine (C) to thymine (T) transition at nucleotide 1715 in both; the mutation encodes an alanine to valine substitution in codon 572 of transmembrane helix 6, A572V. Transfected into COS-7 cells, the A572V mutant exhibited the same constitutively high basal cAMP levels and normal agonist-induced cAMP response as the D578G mutant. We conclude that the constitutively higher cAMP levels caused by the A572V mutation led to Leydig cell activation and male-limited precocious puberty, as in the previously described D578G mutation. As the mother of one of the two patients had the same heterozygous mutation, this patient represents the first recognized case of inherited male-limited precocious puberty in the Japanese population. The previously described D578G mutant did not increase basal or agonist-induced inositol phosphate production in transfected COS-7 cells, or the number of LH/CGRs or their affinity for LH/CG. In contrast, transfection of the A572V mutation in COS-7 cells exhibited significantly higher inositol phosphate levels basally and at 10(-11) mol/L hCG, but significantly lower inositol phosphate levels at 10(-7) mol/L hCG. These data suggest that the A572V mutation of the LH/CGR may have effects on the guanine nucleotide binding protein which activates phospholipase C (Gq) coupling and phospholipase-C activation in addition to its effects on Gs coupling and activation of adenylyl cyclase. A572V-transfected cells also exhibited a higher affinity, despite an apparent decrease in the number of binding sites, for [125I]hCG, compared to transfectants with the wild-type LH/CGR. We hypothesize that these differences between the A572V and D578G mutations reflect a greater impact of the A572V mutation on receptor conformation.


The participation of circulating growth hormone (GH) as a regulator of sex differences in hepatic aldehyde oxidase (AO) activity in ddY mouse was examined. The 2- to 3-fold higher activities in adult male mice compared with adult female mice were decreased to the female levels by neonatal pretreatment with monosodium glutamate (MSG) or monosodium aspartate (MSA), either of which is known to reduce circulating GH levels. A decline of the activities in the MSG-treated male mice was restored nearly to the male control levels by subsequent injections of human GH every 12 hr for 7 days. These changes in AO activities in male mice caused by the excitotoxic amino acids were not observed in females. Hypophysectomy markedly decreased hepatic AO activities in male mice and partially in female mice. The activities in hypophysectomized male mice were restored again to levels similar to the control males by intermittent injections of human GH. Administration of testosterone propionate (TP) significantly increased the activities of hepatic AO in intact female mice, but not in MSA-treated or hypophysectomized females. On the other hand, the AO activities in adult male mice were decreased partially by the administration of estradiol benzoate. These results indicate that the pituitary GH is
involved as one of the major regulatory factors of sex differences in the activities of hepatic AO in mice and TP also contributes to maintaining the higher activity in male mice mainly through the hypothalamus-pituitary system.


INTRODUCTION: Erectile dysfunction is a serious and common complication of diabetes mellitus. Apart from the peripheral actions, central mechanisms are also responsible for the penile erection. AIM: The goal of the present study was to determine the impact of exercise training (ExT) on the centrally mediated erectile dysfunction in streptozotocin (STZ)-induced type I diabetic (T1D) rats. METHODS: Male Sprague-Dawley rats were injected with STZ to induce diabetes mellitus. Three weeks after STZ or vehicle injections, rats were assigned to either ExT (treadmill running for 3-4 weeks) or sedentary groups to produce four experimental groups: control + sedentary, T1D + sedentary, control + ExT, and T1D + ExT. MAIN OUTCOME MEASURE: After 3-4 weeks ExT, central N-methyl-D-aspartic acid (NMDA) or sodium nitroprusside (SNP)-induced penile erectile responses were measured. Neuronal nitric oxide synthase (nNOS) expression in the paraventricular nucleus (PVN) of the hypothalamus was measured by using histochemistry, real time polymerase chain reaction (PCR) and Western blot approaches. RESULTS: In rats with T1D, ExT significantly improved the blunted erectile response, and the intracavernous pressure changes to NMDA (50 ng) microinjection within the PVN (T1D + ExT: 3.0 +/- 0.6 penile erection/rat; T1D + sedentary: 0.5 +/- 0.3 penile erection/rat within 20 minutes, P < 0.05). ExT improved erectile dysfunction induced by central administration of exogenous nitric oxide (NO) donor, SNP in T1D rats. Other behavior responses including yawning and stretching, induced by central NMDA and SNP microinjection were also significantly increased in T1D rats after ExT. Furthermore, we found that ExT restored the nNOS mRNA and protein expression in the PVN in T1D rats. CONCLUSIONS: These results suggest that ExT may have beneficial effects on the erectile dysfunction in diabetes through improvement of NO bioavailability within the PVN. Thus, ExT may be used as therapeutic modality to up-regulate nNOS within the PVN and improve the central component of the erectile dysfunction in diabetes mellitus.