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INFLUENCE OF GENDER AND BRAIN REGION ON NEUROSTEROID MODULATION OF GABA RESPONSES IN RATS

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Summary

Neuroactive steroid derivatives of progesterone, testosterone and glucocorticoids can alter physiological responses to γ-aminobutyric acid (GABA), apparently through direct, non-steroid receptor mechanisms. The present study examined gender-related differences and regional variations in the ability of tetrahydrodeoxycorticosterone (THDOC), 3α-hydroxy-5α-pregnan-20-one (3α-5α-THP, tetrahydroprogesterone), androsterone, and dihydroandrosterone (DHA) to alter physiological GABA responses. Steroid modulation of GABA-activated ^{36}chloride influx into microsac preparations from cortex, hippocampus, amygdala, cerebellum and hypothalamus-preoptic area in adrenalectomized-gonadectomized rats of both sexes were tested. The effects of THDOC and 3α-5α-THP were also examined in groups of intact male and female rats. All four steroids increased GABA-activated chloride influx, although the maximal enhancement in GABA responses differed significantly among brain regions. The rank order of maximal THDOC and 3α-5α-THP effects was hippocampus > cortex ~ amygdala > hypothalamus-preoptic area ~ cerebellum. Regional differences in potentiation of GABA responses were seen with androsterone, but not dihydroandrosterone. The rank order of androgenic potentiation of GABA responses was amygdala ~ hippocampus > cortex ~HPA > cerebellum. Slight gender-related differences in responses to steroids were seen with THDOC, with males showing greater maximal enhancement of GABA responses with THDOC than females in the amygdala and hypothalamus-preoptic area. Since sex differences were observed with the glucocorticoid derivative THDOC, but not the progesterone derivative 3α-5α-THP or androgenic steroids, it appears neuroactive steroid modulation of GABA responses can be differentially affected by the hormonal milieu in a regionally-specific manner.

Key Words: γ-aminobutyric acid (GABA), sex differences, regional differences, neurosteroid, tetrahydrodeoxycorticosterone, tetrahydroprogesterone, androgen, chloride influx

Steroid derivatives of progesterone, testosterone and glucocorticoids can alter physiological responses to γ-aminobutyric acid (GABA), apparently through direct, non-steroid receptor mechanisms (1-7). Several of these steroid metabolites have consistently been shown to enhance

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GABA functions through direct interaction with a distinct site within the GABA receptor complex. These include the reduced progesterone metabolite 3α-hydroxy-5α-pregnan -20-one (3α-5α-THP), the glucocorticoid derivative tetrahydrodeoxycorticosterone (THDOC), and the androgenic steroids 5α-androstan-3α-ol-17-one (androsterone) and 5α-androstan-3α,17β diol (dihydroandrosterone, androstanediol). When added directly to *in vitro* assays, such steroids enhance BZ binding, inhibit channel-site t-butylbicyclophosphorothionate (TBPS) binding, and potentiate GABA-activated chloride flux into synaptosomes (1,2,8,9). Direct, local applications of steroids also affect various physiological indices of GABA function with a very rapid time course (1-7). Neuroactive steroids, especially 3α-5α-THP and THDOC, can potentiate the inhibitory actions of GABA in cultured neurons (1,2,10) or adrenal chromaffin cells (5) *in vitro*, and enhance GABA currents expressed in oocytes injected with brain RNA (11). The rapid effects of neuroactive steroids on physiological responses to GABA, combined with their *in vitro* modulation of receptor sites, suggests that these derivatives interact directly with the GABA/BZ/ionophore complex. Studies with recombinant GABA receptors indicate that neuroactive steroid effects can be modified by the subunit structure, predominantly by changes in the α and γ subunits (10,12,13).

Further, these neuroactive steroids have behavioral actions or actions *in vivo* consistent with their ability to potentiate GABAergic processes and reminiscent of the benzodiazepines. The sedative/ anesthetic properties of progesterone and other steroid derivatives are well known (14). Anxiolytic effects of progesterone and neuroactive steroids have also been demonstrated, and the anxiolytic effects of progesterone are significantly correlated with levels of its neuroactive steroid metabolite 3α-5α-THP (15-17). Similarly, heightened levels of progesterone and administration of 3α-5α-THP are associated with protective (anticonvulsant) effects against seizures (18,19). The most conclusive demonstration that neuroactive steroids can modify GABAergic responses *in vivo* comes from the physiological studies of Smith and colleagues in cerebellum (7). In anesthetized ovariectomized (OVX) rats, progesterone administration rapidly potentiates the GABA-evoked inhibition of cerebellar Purkinje cells (7). Several lines of evidence indicate that these effects are mediated through the conversion of progesterone to its neuroactive steroid metabolite 3α-5α-THP (7). Systemic (i.v.) administration of androgenic steroids (androsterone and androstanediol) also rapidly (within seconds) decreased multiunit activity, blocked epileptiform activity, and altered EEG activity in several brain regions (20).

A few studies have indicated that these neuroactive steroid effects on GABAergic systems may have some degree of regional specificity (21-24). These studies have utilized the ability of neuroactive steroids to modify binding parameters of GABA receptors sites, including the channel-related TBPS site (21,22,24) or the GABA receptor site labeled with muscimol (23). Studies using neuroactive steroid modulation of TBPS binding have also indicated both sex differences and influences of estrous cycle on neuroactive steroid potency (24). The present study examined gender related differences and regional variations in the ability of four neuroactive steroids or "neurosteroids" to alter physiological GABA responses. The ability of THDOC and 3α-5α-THP to modulate GABA-activated ^{36}chloride influx into synaptoneurosomal preparations was analyzed in five brain areas from intact or adrenalectomized-gonadectomized (ADX-GNX) male and female rats. Since several studies have indicate that androgenic compounds can affect GABAergic responses (22,25,26), the effects of two androgenic neuroactive steroids (androsterone and dihydroandrosterone) were also examined in groups of ADX-GNX male and female rats. Enhancement of physiological GABA responses with THDOC, 3α-5α-THP, and androsterone showed significant regional variation, while gender-related differences in responses to steroids were only seen with THDOC in the amygdala and hypothalamus-preoptic area.

Methods

Subjects.

Male and female Long Evans hooded rats (Harlan Sprague Dawley Inc, Indianapolis IN) were group housed with food and water available *ad libitum*. Adrenalectomized animals were maintained on saline drinking water. Animals were maintained in an AAALAC accredited facility under a 12:12 hr light:dark cycle, with sacrifice occurring during the early light period (3 hrs after lights on). Protocols were approved by the University of South Carolina's Institutional Care and Use Committee. Rats were 2-3 months of age at the time of sacrifice. Animals were habituated to the procedure eventually used for decapitation to diminish the influences of stress on GABA responsiveness.

Two groups of animals were tested for their responses to the neuroactive steroids THDOC and 3α-5α-THP in various brain regions. Groups of adrenalectomized-orchidectomized male (ADX-ORCH) and adrenalectomized-ovariectomized female (ADX-OVX) rats were tested to minimize the influences of endogenous steroid levels. Adrenalectomy-gonadectomy (ADX-GNX) surgery was performed by the vendor 2-3 weeks prior to testing, when rats were 1.5 -2 months of age. Groups of ADX-GNX rats were also tested for their responses to the androgenic derivatives androstanediol and dihydroandrosterone. Additional groups of intact male and cycling female rats were tested for their responses to THDOC and 3α-5α-THP. Females were sacrificed during diestrous which was determined using vaginal smears and confirmed by uterine weights. Verification of adrenal removal was determined using radioimmunoassay of serum corticosterone levels (see (27) for methods) and gonadectomy was confirmed by visual inspection (males) and uterine weights (females). Intact animals were not tested for androgen responses, due the minimal influences of these steroids on GABA activated chloride influx in ADX-GNX rats.

Animals were sacrificed by decapitation, and the brains rapidly removed. The cerebral (parietal) cortex, the hippocampus, the amygdala, the hypothalamus-preoptic area (HPA), and the cerebellum from each animal were immediately dissected on ice. Tissue from two (cortex, cerebellum) or three (amygdala, hippocampus, HPA) rats were pooled to obtain sufficient tissue for analysis of two steroids on a single microsac preparation. Results are from 3-5 separate microsac preparations for each neuroactive steroid.

Analysis of Chloride Flux

Determination of GABA stimulation of $^{36}Cl^-$ into brain vesicles was used as a biochemical measure of physiological responses to GABA. GABA-stimulated chloride flux was analyzed using a modified method (28) of Harris and Allan (29). Samples were homogenized in 70 volumes cold buffer (w/v) by hand with 12 strokes in a glass-Teflon homogenizer. Buffer consisted of 10 mM HEPES plus 145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 10 mM glucose, 1 mM $CaCl_2$ (pH 7.5). Samples were centrifuged at 1000 x g (15 min, 4°C). The pellet was resuspended by vortexing in 70 volumes cold buffer and centrifuged at 1000 x g (15 min). Pellet was resuspended to yield 7 mg/ml protein (10 volumes/tissue weight) and aliquots (150 μl) were incubated at 30°C for 5 min with neuroactive steroids. Tubes containing buffer rather than sample were used as blanks, and blank values were subtracted from all samples. A solution of $^{36}Cl^-$ (1.5 μCi/ml buffer) with 10 μM GABA (200 μl) was added to the tubes and immediately vortexed, thus initiating $^{36}Cl^-$ uptake. Three seconds later, $^{36}Cl^-$ influx was terminated by addition of 4 ml ice-cold buffer containing picrotoxin (300 mg/l) to help stop the reaction. Samples were immediately filtered over glass microfiber filters (GF/C) using a Hoefer Filtration Apparatus (Hoefer Scientific). Filters were washed once with 4 ml picrotoxin buffer and then with 6 ml assay buffer. $^{36}Cl^-$ taken up into the

"microsacs" was determined using liquid scintillation spectrophotometric techniques (Beckman 6000 IC Scintillation counter). The effects of the neuroactive steroids THDOC, 3α-5α-THP, androsterone, and dihydroandrosterone (DHA) were tested by pre-incubating duplicate sample aliquots with 4 doses (100-3000 nM; final concentration) of steroid at 30oC for 5 min and assessing the ^{36}chloride influx in the presence of 10 μM GABA. Steroids were pre-incubated with tissue as described in (1), since preliminary experiments indicated results were similar whether steroids were preincubated with tissue or added with the chloride solution. Further, preincubation of steroids with the tissue tended to yield more reproducible results and might more accurately reproduce the in vivo exposure to neuroactive steroids, which probably occurs over a longer time period than 3 seconds. A pooled membrane preparation from 2-3 rats was used to permit comparison of two neuractive steroids on the same membrane preparation, although due to the small tissue size in some areas only duplicate samples could be analyzed. Basal samples at 10 μM GABA were run in quadruplicate. In order to minimize the number of animals required, we enhanced the number of separate microsac preparations analyzed, rather than increase the replicates per preparation. GABA concentration was held at 10μM for all areas, since other studies have indicated that concentration is below the GABA EC_{50} in these regions, which would permit the observation of greater that 50 percent potentiation in all brain regions. Protein values of samples were determined using the Lowry et al. assay (30) and influx results are reported as nmols total Cl/3 sec/mg protein or as the percent increase over chloride flux seen with 10 μM GABA alone.

Materials

Steroids used in this study were: tetrahydrodeoxycorticosterone (5α-pregnan-3α,21-diol-20-one, THDOC; Steraloids, Inc., Wilton, NH; P2560), 5α-pregnan-3α-ol-20-one (3α5α-THP; Sigma P8887, St. Louis MO), androsterone (5α-androstan-3α-ol-17-one, Sigma A-9755, St. Louis MO) and dihydroandrosterone (5α-androstane-3α,17β-diol, 3α-androstanediol; DHA, Sigma A-7755, St. Louis MO). Steroids were diluted with assay buffer from a stock solution in ethanol, such that final alcohol concentration in the assay was less than 0.1%, and chloride influx at 10 μM GABA was analyzed in the presence of similar alcohol vehicle solution. ^{36}Chlorine was purchased from Dupont New England Nuclear (NEZ019; 13 Ci/gm.)

Statistics

The effects of steroids are expressed as the percent increase in GABA-activated ^{36}chloride flux, calculated as the steroid-induced difference divided by the response seen with 10 μM GABA alone. Statistical analysis was performed separately on the results from each of the four steroids. Steroid dose response curves for brain regions were compared using Analysis of Variance (ANOVA) with repeated measures design (dose of steroid). Hormone groups were compared within each brain region using ANOVA (hormone group) with repeated measures (dose of steroid), with male-female comparisons and ADX/GNX effects analyzed by ANOVA contrast analysis (SAS, Cary NC). For the level of GABA-stimulated ^{36}chloride flux at 10 μM GABA (no steroid) differences between hormone groups were assessed using ANOVA in each brain area, with contrast analysis to determine male-female and intact versus ADX-GNX differences. ANOVA was also used to assess differences between brain regions, with values collapsed across hormone groups.

Results

THDOC: The basal levels of GABA-activated ^{36}chloride influx observed at 10 μM GABA (in the absence of any steroid) were not affected by gender in either the intact or ADX-GNX experiments (see TABLE I). Increasing doses of THDOC increased GABA activated ^{36}chloride

TABLE I

No Sex Differences were seen in Chloride Flux at 10 μM GABA

	Amygdala	Cortex	Hippo	HPA	Cerebellum
Intact Female	23 ± 1	24 ± 1	20 ± 1	14 ± 1	21 ± 1
Intact Male	23 ± 2	23 ± 2	20 ± 0.4	14 ± 1	20 ± 0.1
ADX-OVX	30 ± 3	29 ± 2	21 ± 3	15 ± 1	23 ± 0.2
ADX-ORCH	28 ± 3	29 ± 3	21 ± 2	16 ± 2	25 ± 1

The level of chloride flux at 10 μM GABA was not significantly altered by hormonal status. Values show mean ± S.E.M. nmol chloride/3 sec/mg protein of 5 separate microsac preparations.

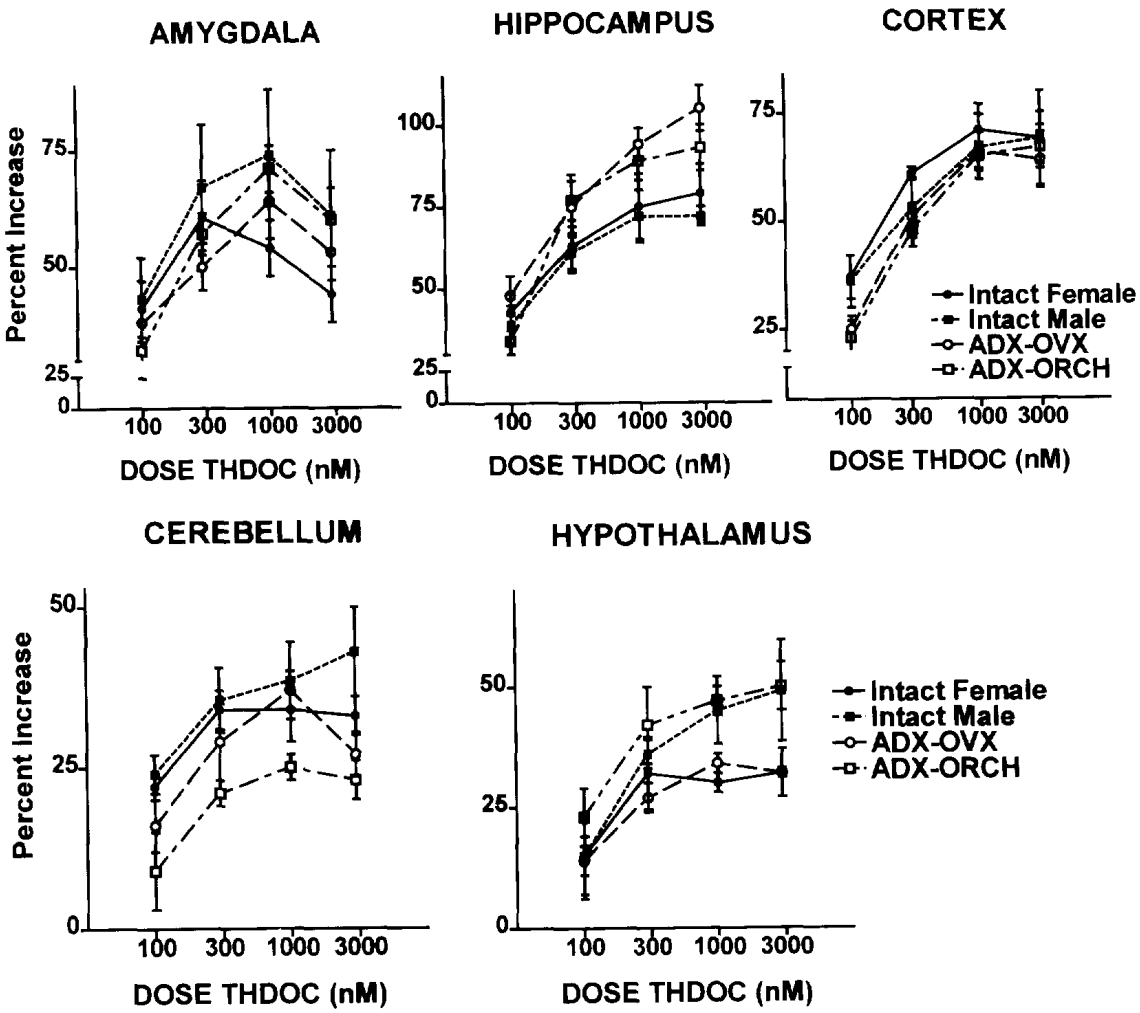

Fig. 1.

Dose response curves showing the percent increase in GABA-activated chloride flux with THDOC in intact males, intact females, adrenalectomized-orchidectomized (ADX-ORCH), and adrenalectomized-ovariectomized (ADX-OVX) groups. The rank order of maximal THDOC effects was hippocampus > cortex ~ amygdala > hypothalamus-preoptic area ~ cerebellum. Gender differences in THDOC effects were seen in amygdala and HPA. Note that graphs from HPA and cerebellum are represented using a different scale than other brain regions, due to the reduced level of enhancement seen in these areas. Each point represents the mean ± S.E.M. of 5 separate microsac preparations from each brain area.

influx in all brain regions (see Fig.1; $F_{(3,92)}=213$; $P<0.0001$ for dose effect using repeated measures ANOVA). Brain regions differed significantly in their responses to THDOC in both intact ($F_{(4,41)}=11$, $P<0.0001$) and ADX-GNX ($F_{(4,43)}=47$, $P<0.0001$) animals. As seen in Fig. 2, the rank order of maximal THDOC effects was Hippocampus > Cortex ~ Amygdala > Hypothalamus-preoptic area ~ Cerebellum. Although brain areas also differed in chloride influx at 10 μM GABA (see TABLE I, $F_{(4,40)}=13$, $P<0.0001$), regional THDOC responses differed similarly whether results were expressed as percent change (Fig. 1) or as nmol/mg protein of chloride influx.

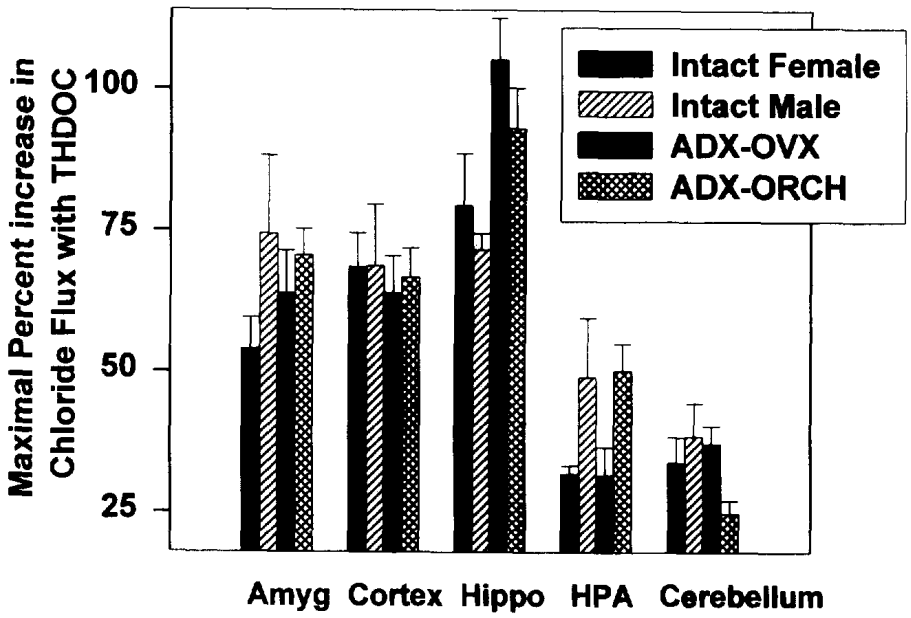

Fig. 2

The maximal percent increases in GABA-activated chloride flux observed with THDOC in each group for each brain area are shown. Maximal THDOC effects were greatest in hippocampus > Cortex ~ Amygdala > Hypothalamus-preoptic area (HPA) ~ Cerebellum. Areas also show differences in sensitivity to THDOC, since the maximal effects were seen at 1000 nM concentration in amygdala and cerebellum, and 3000 nM in other brain areas. Males showed significant greater increases with THDOC than females in HPA and amygdala, although this sex differences was not observed in gonadectomized animals in the amygdala. GNX-ADX groups also showed significantly greater increases in GABA responses with THDOC than intact groups in the hippocampus. Each bar represents the mean ± S.E.M. of 5 separate microsac preparations from each brain area.

Gender-related differences in dose-related responses to THDOC were seen in the amygdala and hypothalamus (see Fig . 1 and 2). In the amygdala, maximal effects of THDOC occurred at low doses relative to other brain areas, and high doses of THDOC tended to depress levels of GABA activated ^{36}chloride influx (see Fig. 1). The dose response curves for THDOC in the amygdala were significantly affected by hormonal status ($F_{(9,45)}=3.0$, $P<0.01$), with intact males showing greater GABA-activated chloride influx than intact females at high doses of THDOC ($F_{(1,24)}=3.4$ for dose by sex interaction, $P<0.025$) As seen in Fig. 2, this difference was not observed in ADX-GNX groups ($F=0.25$, $P=0.6$ for sex effect and $F=2$, $P=0.2$ for dose by sex interaction). Similarly,

in the hypothalamus males also showed greater increases in GABA activated chloride influx at high THDOC doses than female ($F_{(1,24)}$=5.4 $P<0.05$ for gender effect). In cerebellum, THD0C-induced increases were diminished in the ADX-ORCH group when compared with values from ADX-OVX, intact male, or female groups, although this gender differences in ADX-GNX groups failed to attain statistical significance ($F_{(1,24)}$=4.4, $P=0.07$).

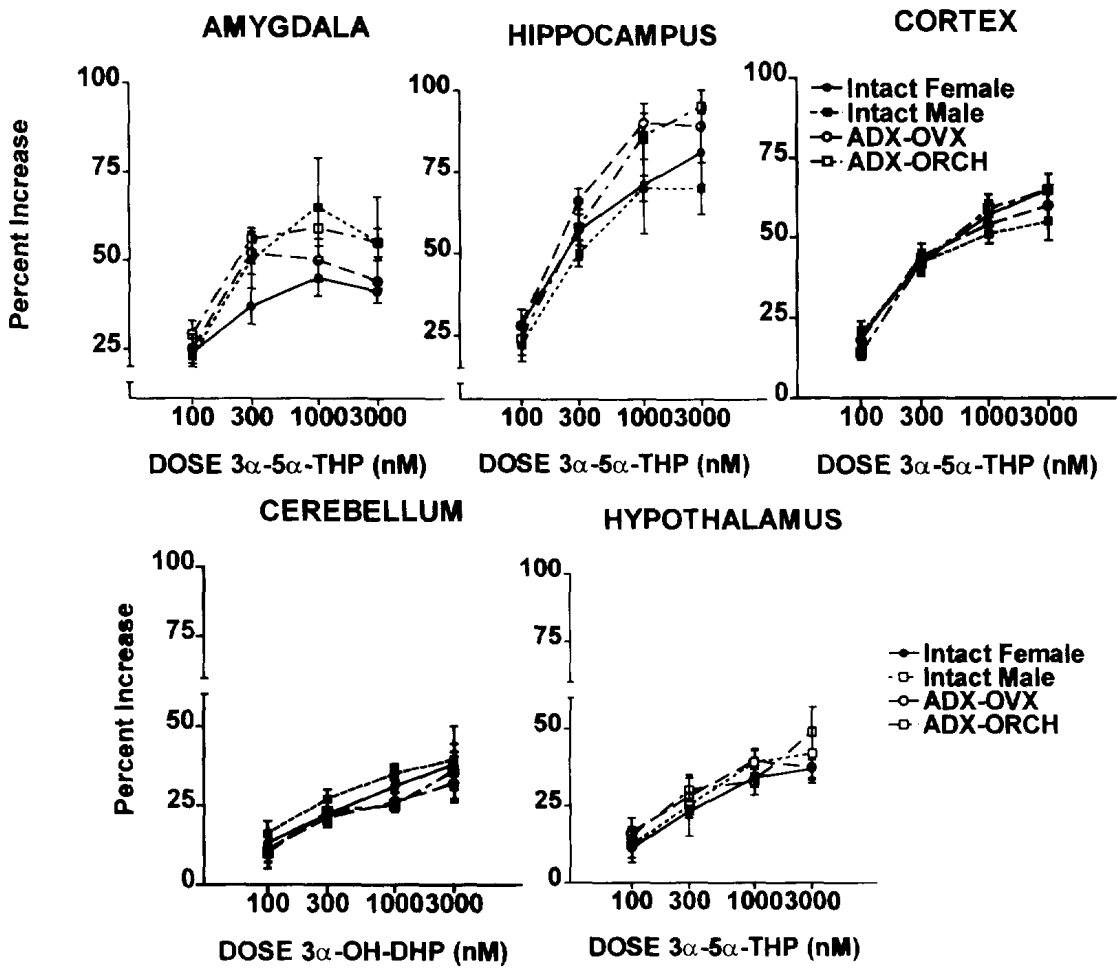

Fig. 3.

Dose response curves showing the percent increase in GABA-activated chloride influx with 3α-5α-THP in intact males, intact females, adrenalectomized-orchidectomized (ADX-ORCH), and adrenalectomized-ovariectomized (ADX-OVX) groups. The rank order of maximal effects was Hippocampus > Cortex ~ Amygdala > Hypothalamus-preoptic area ~ Cerebellum. No gender-related differences in 3α-5α-THP effects were seen. Note that graphs from HPA and cerebellum are represented using a different scale than other brain regions, due to the reduced level of enhancement seen in these areas. Each point represents the mean ± S.E.M. of 5 separate microsac preparations from each brain area.

3α-5α-THP: Increasing doses of 3α-5α-THP increased GABA activated ^{36}chloride influx in all brain regions (See Fig. 3; $F_{(4,92)}=307$; $P<0.0001$ for dose effect using repeated measures ANOVA). Brain regions differed significantly in their responses to 3α-5α-THP, with the rank order of maximal effects being Hippocampus > Cortex ~ Amygdala > Hypothalamus-preoptic area~Cerebellum. Using repeated measures analysis, areas differed significantly in their responses to 3α-5α-THP in both intact ($F_{(4,41)}=47$, $P<0.0001$) and ADX-GNX ($F_{(4,43)}=47$, $P<0.0001$) animals. No significant gender-related differences were observed in responses to 3α-5α-THP in any brain region (see Fig. 3). In amygdala, maximal effects of 3α-5α-THP appeared greater in males than females, although this differences was only evident at high doses and was not statistically significant.

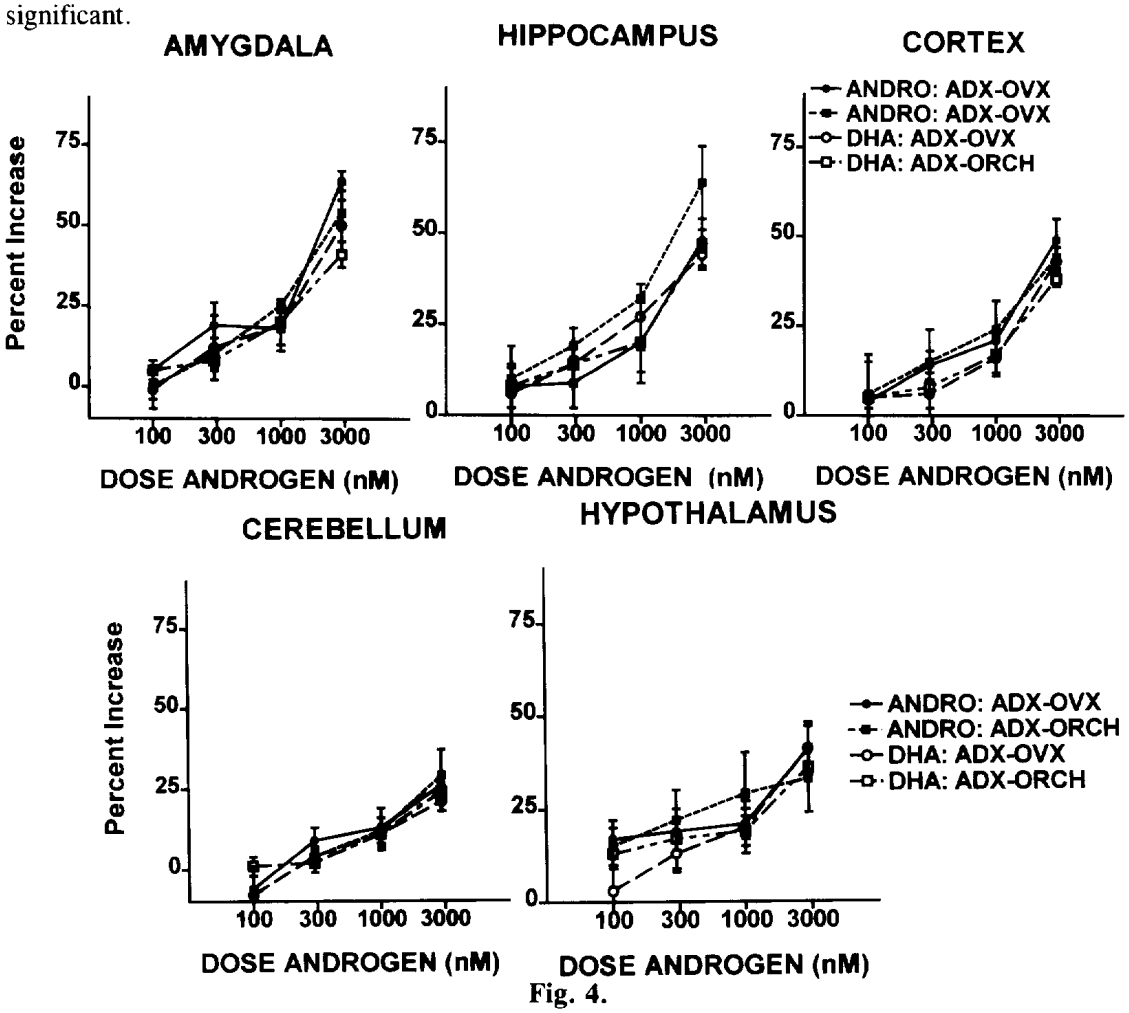

Fig. 4.

Dose response curves showing the percent increase in GABA-activated chloride influx with androsterone (ANDRO) and dihydroandrosterone (DHA) in adrenalectomized-orchidectomized (ADX-ORCH), and adrenalectomized-ovariectomized (ADX-OVX) groups. The rank order of maximal effects of androsterone was Amygdala~ Hippocampus> Cortex~ Hypothalamus-preoptic area > Cerebellum. No gender-related differences in androgen effects were seen. Each point represents the mean ± S.E.M. of 3 separate microsac preparations from each brain area.

Androgens: Both androsterone ($F_{(3,100)}=188$, $P<0.0001$) and dihydroandrosterone (DHA; $F_{(3,100)}=178$, $P<0.0001$) increased GABA-activated ^{36}chloride influx in a dose-related fashion in

ADX-GNX rats (Fig. 4). The magnitude of these increases were substantially less than those observed with THDOC or 3α-5α-THP, and generally seen only at the higher doses of the steroid. The dose response curves for androsterone ($F_{(4,25)}=4$, $P<0.01$), but not DHA ($F_{(4,25)}=2$, $P=0.14$), differed significantly between brain areas. The rank order for maximal increases with androsterone was amygdala ˜ hippocampus > cortex ˜HPA > cerebellum. In ADX-GNX rats, no gender-related differences were observed in any brain area for androsterone and DHA (Fig. 6). Since GABA responses did not show increases above basal levels except with high doses of androgen, similar analyses were not performed in intact hormone groups.

Discussion

The present study examined gender-related differences and regional variations in the ability of THDOC, 3α-5α-THP, androsterone, and dihydroandrosterone (DHA) to alter physiological GABA responses. Neuroactive steroid modulation of GABA-activated ^{36}chloride influx into microsac preparations from cortex, hippocampus, amygdala, cerebellum and hypothalamus-preoptic area (HPA) was compared in adrenalectomized-gonadectomized (ADX-GNX) male and female rats. The effects of THDOC and 3α-5α-THP were also examined in groups of intact male and female rats. Marked regional differences were observed in the ability of THDOC, 3α-5α-THP, and androsterone to enhance physiological GABA responses. The rank order of maximal THDOC and 3α-5α-THP effects was Hippocampus > Cortex˜Amygdala > Hypothalamus-preoptic area˜Cerebellum. Regional differences in androgenic steroid effects were seen with androsterone, with a rank order of amygdala ˜ hippocampus > cortex ˜HPA > cerebellum, while regional differences were not seen with dihydroandrosterone. Slight gender-related differences in responses to steroids were seen only with THDOC in the amygdala and hypothalamus-preoptic area.

All four steroids tested increased GABA-activated chloride influx, although the maximal increases in GABA-activation of chloride influx differed among brain regions. These results support and extend previous studies showing regional differences in the ability of neuroactive steroids to modify GABA receptor sites using receptor binding analyses (21-24). In ovariectomized females, systemic administration of progesterone, but not 3α-5α-THP, increased GABA receptors (^{3}H-muscimol binding) in cortex and hippocampus and increased the affinity of GABA receptors in hypothalamus (23). The effects of the neuroactive steroid anesthetic alphaxalone and 3α-5α-THP on {^{35}S}TBPS-labeled channel sites also differed among brain areas (22,24). Previous studies have indicated that cerebellum appears to show greater effectiveness and increased sensitivity to several neuroactive steroids using analysis of TBPS binding (22,24). The present results, however, indicate that both 3α-5α-THP and THDOC induced relatively small effects on GABA-activated chloride influx in cerebellum (25-40%), at least when compared with cortex, hippocampus and amygdala (> 50% maximal increase). These differences may be partially related to methodological considerations and/or to differences between analyzing a binding site and a physiological response. In general, our results support the notion that neuroactive steroid regulation of GABA receptors and their physiological responses is regionally specific, and the cerebellum may differ considerably from the other brain areas (22,24).

These regional differences in neuroactive steroid influences on GABA responses could be related to variant patterns of GABA receptor subunit expression. For example, the low level of responsiveness to 3α-5α-THP and THDOC in cerebellum may be related to the distinctive expression of α6 GABA receptor subunits in this region (31). Prior studies have suggested that alterations in the α and γ subunits can alter neuroactive steroid sensitivity of recombinant GABA receptors, and generally receptors containing the α1 subunit display greater neuroactive steroid activation of GABA-

activated chloride currents than receptors containing the α6 subunit (10,12,13,32), and neuroactive steroid modulation of TBPS binding also differed between receptors containing these two subunits (33). Further, the ability of 3α-5α-THP to modulate GABA receptor binding sites appears biphasic in receptors with the α6 subunit, but not in receptors containing the α1 subunit (34).

The effects of 3α-5α-THP and THDOC enhancement of GABA-induced chloride influx were seen with doses generally similar to those reported earlier for studies analyzing GABA-activated chloride influx (~100-300 nM) and steroid regulation of GABA receptor binding sites, although some studies report that the potency of these steroids is less than 100 nM (1,2,8,9,21,22,24,34-36). Interestingly, amygdala appeared to display a greater sensitivity for 3α-5α-THP and THDOC enhancement of GABA responses than other brain regions, and a biphasic response to these steroids. For example, prior studies comparing various brain areas have reported IC_{50} values of approximately 1.5 - 10 μM for 3α-5α-THP or THDOC inhibition of TBPS binding (21,24). Electrophysiological studies in hippocampal slices have indicated that local application of an estimated dose of 200 nM 3α-5α-THP depressed the evoked population spike amplitude from CA1 pyramidal cells (37). Studies have also indicated that high (μM) concentrations of these steroids can accelerate desensitization of GABA-activated chloride channels and inhibit GABA responses in recombinant receptors (34). Expression of rat cortex poly(A)$^+$RNA, however, suggests that doses of 1-500 nM 3α-5α-THP or THDOC potentiate GABA effects without directly increasing chloride currents, although rate of desensitization was increased by high steroid doses (11). This could suggest that the preincubation with neuroactive steroids could have induced enhanced desensitization of GABA responsiveness, and could account for some of the region- and gender-specific changes observed in the present study. Generally, the ability of the androgenic steroids to enhance GABA responses required much higher doses (1000-3000 nM) than THDOC or 3α-5α-THP (22,25). Although the sensitivity to 3α-5α-THP, THDOC, and the androgens appear similar to those seen using other *in vitro* assays of GABA receptor regulation or responses, measured brain concentrations of these steroids do not appear to approach these values. For example, 3α-5α-THP levels in a "normal" male are~2-4 nM (38), although levels of metabolites may increase to 10-20 nM after stress in rodents (32,38-40). Stress also increases cortical levels of THDOC (up to 1 ng/g; (39)) and circulating levels of DHA (26). Since analysis of GABA-activated chloride flux was performed on crudely dissected brain areas, it is possible that certain cell populations within a region are much more sensitive to neuroactive steroid effects or that neuroactive steroid concentrations may be higher in localized areas or near membrane fractions.

Although steroid derivatives such as DHA and THDOC appear to arise from predominantly peripheral (adrenal or gonadal) sources, levels of some progesterone derivatives are not completely eliminated from brain by adrenalectomy-gonadectomy (26,32,38,39,41). The latter finding suggests that the brain is capable of synthesizing some neuroactive steroids (7,32,42). Levels of progesterone and progesterone metabolites, as well as the enzymes needed for the derivitization of neuroactive steroids, also show regional variations (40,43,44). Studies suggest that the ability to synthesize various steroid derivatives can be specific to certain brain nuclei (45) and may indicate high concentrations of neuroactive steroids could be in restricted to selective brain loci. The mRNA for cytochrome P450scc is absent in cerebellum and hypothalamus (43), which are the two regions showing a reduced potentiation of GABA responses with THDOC and 3α-5α-THP. Cytochrome P450c11β mRNA has also been found in neurons in a regionally specific manner, suggesting that the brain may have capability of synthesizing glucocorticoids (43). Some suggest that the mitochondrial peripheral BZ receptor may play some role in regulating synthesis of steroids and neuroactive steroids (46).

Although gender and reproductive status can affect levels of progesterone derivatives (32,38,47) and steroid synthetic enzymes (40,47), only slight sex differences in THDOC responses were observed in the present study. Females showed slightly reduced THDOC responses compared with males in amygdala and hypothalamus. Similarly, differences between males and diestrus females have been observed in the potency of THDOC-induced inhibition of cortical TBPS binding (24) and cytochrome P450c11β mRNA in hippocampus was reliably greater in females than males (43) . Adrenalectomized-gonadectomized groups tended to be less sensitive to THDOC than intact groups in the hippocampus, and showed no gender-related differences in amygdala THDOC responses, perhaps suggesting that the removal of peripheral sources of THDOC might be shifting sensitivity slightly from that observed in hormonally (adrenally)-intact groups. This would be consistent with the possibility that some low level of THDOC in the membrane preparation and was shifting the dose response curve for THDOC. This notion is supported by results using TBPS binding indicating that male-female differences in THDOC potency were dependent upon how well the membrane preparation had been washed (24). While this remains a possibility, a similar trend was not observed with 3α-5α-THP in the same tissue preparations and was not seen to the same degree in all brain regions. Alternatively, these results might suggest that ADX-GNX alters the sensitivity of GABA receptor responses to modulation by THDOC. This might be consistent with the possibility that this glucocorticoid derivative plays some role in modulating neuronal circuits associated with feedback from glucocorticoids.

In conclusion, marked regional variations were observed in the ability of THDOC, 3α- 5α-5α-THP, and androsterone to enhance GABA responses. Limbic areas, such as hippocampus, amygdala and cortex, displayed greater maximal responses to these steroids than hypothalamic or cerebellar preparations. These results further suggest that the ability of THDOC and 3α-5α-THP to potentiate GABA responses can be differentially affected by the hormonal milieu, since gender-related differences in neuroactive steroid modulation of GABA responses were observed with the glucocorticoid derivative THDOC, but not the progesterone derivative 3α-5α-THP or androgenic steroids.

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