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ORIGINAL INVESTIGATION

Scott Wieland · James D. Belluzzi · Larry Stein
Nancy C. Lan

Comparative behavioral characterization of the neuroactive steroids 3α-OH,5α-pregnan-20-one and 3α-OH,5β-pregnan-20-one in rodents

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Abstract Pregnan steroids have been shown to possess anesthetic, hypnotic, anticonvulsant and anxiolytic properties. In this study, two endogenous neuroactive steroid isomers, 3α-hydroxy-5α-pregnan-20-one (3α,5α-P) and 3α-hydroxy-5β-pregnan-20-one 3α,5β-P), were studied for differences in their pharmacological properties using behavioral assays. 3α,5α-P and 3α,5β-P were similar in their potencies and efficacies in blocking pentylenetetrazol-induced seizures in mice (ED_{50}: 3α, 5α-P = 2.8 mg/kg and 3α,5β-P = 3.0 mg/kg). Similarly, both neuroactive steroids produced roto-rod deficits within the same range of potency (TD_{50}: 3α,5α-P = 18.8 mg/kg and 3α,5β-P = 21.2 mg/kg). However, in animal models of anxiety, subtle differences were observed between the two isomers. In both the light/dark transition test and elevated plus-maze, 3α,5β-P was more efficacious than 3α,5α-P, though both compounds had similar potencies. In the Geller-Seifter test, 3α,5β-P was more potent and efficacious than 3α,5α-P. Neither compound had significant effects on unpunished responding within the dose range tested. Both compounds produced similar biphasic curves in the locomotor test. All together, the data indicate that 3α,5α-P and 3α,5β-P have similar anticonvulsant activity, but the 5β-isomer possesses more potent and efficacious anxiolytic properties than the 5α-isomer.

Key words Neuroactive steroids · Anxiolytic Geller-Seifter · 3α, 5α-P · 3α, 5β-P

Scott Wieland (✉) · Nancy C. Lan
CoCensys, Inc., 213 Technology Drive, Irvine, CA 92718, USA

James D. Belluzzi · Larry Stein
Department of Pharmacology, University of California, Irvine, Irvine, CA 92717, USA

Introduction

$GABA_A$ receptors are ligand-gated Cl^- channel complexes (GRC) which are involved not only in the major neurotransmitter GABA, but also the pharmacological actions of therapeutic drugs such as benzodiazepines (BZ) and barbiturates. Recently, neuroactive steroids were shown allosterically to modulate GABA through a unique binding site on the GRC (Gee 1988). The mechanism by which neuroactive steroids modulate GABA was found to be distinctly different from that of the benzodiazepines and the barbiturates using molecular (Lan et al. 1990; Puia et al. 1990), biochemical (Majewska et al. 1986; Gee 1988; Gee et al. 1988; Morrow et al. 1990), and electrophysiological (Peters et al. 1988) techniques.

Both endogenous and synthetic neuroactive steroids have been shown to be potent anticonvulsant, anxiolytic, sedative/hypnotic and anesthetic agents. The progesterone metabolite 3α-OH-5α-pregnan-20-one (3α,5α-P) has been shown to be a potent anticonvulsant (Belelli et al. 1989) and anxiolytic in the Vogel test (Wieland et al. 1991), light/dark transition test (Wieland et al. 1991), and elevated plus-maze (Bitran et al. 1991). The deoxycorticosterone metabolite 5α-pregnan-3α,21α-diol-20-one (5α-THDOC) has been shown to attenuate stressed-induced increases in plasma corticosterone (Owens et al. 1992), produce anxiolytic effects in the light/dark transition test (Crawley et al. 1986) and Vogel test (Crawley et al. 1986). In addition, 5α-THDOC has been shown to produce sedative/hypnotic effects (Mendelson et al. 1987) and reduce aggressive behavior (Kavaliers 1988). The synthetic pregnan-related neuroactive steroid anesthetic, alphaxalone, has been shown to increase responding in the conflict portion of the Geller-Seifter test and produce anxiolytic-like effects in the elevated plus-maze test without any analgesic effects (Britton et al. 1991).

The 5β isomer of the endogenous neuroactive steroid 3α,5α-P is known to also exist naturally. 3α,5β-P has been shown to be as potent as 3α,5α-P in the allosteric modulation of GABA (Gee and Lan 1991). However, there have been few published data on the pharmacological differences between these two endogeneous isomers. Bitran et al. (1991) showed that 3α,5β-P produced anxiolytic-like effects in the elevated plus-maze when injected into the cerebroventricles of female rats. The results from this study suggested that 3α,5α-P was more potent than 3α,5β-P, but it was less efficacious. Since both 3α,5α-P and 3α,5β-P are insoluble in most vehicles, it has been difficult to compare results between studies. The current study was designed to compare the two neuroactive steroids in parallel using 20% 2-hydroxypropyl-β-cyclodextrin as a vehicle. This vehicle solubilizes both neuroactive steroids and provides a homogeneous solution for parental administration. A series of behavioral assays was used to determine whether there are subtle differences in the behavioral responses between 3α,5α-P and 3α,5β-P. Both compounds were tested for their ability to block pentylenetetrazol-induced seizures and their sedative effects as measured using the roto-rod test in mice. In addition, they were tested in three animal models of anxiety including the light/dark transition test in mice, elevated plus-maze in mice, and the Geller-Seifter conflict test in rats. Pharmacological effects on locomotor activity were also measured in mice.

Materials and methods

Animals

Male CF/1 mice (Charles River, Wilmington, Moss.) weighing 20–30 g were used in the anti-pentylenetetrazol, roto-rod, and locomotor activity studies. Male NIH Swiss-Webster mice (Harlan, Indianapolis, Ind.) weighing 20–30 g were used in the light/dark transition and elevated plus-maze tests. Animals were grouped housed (n = 4/cage) in a light (0600–1800 hours and temperature (22°C) controlled vivarium. Food and water were available ad libitum, except during testing. The experiments were run from (0700 to 1500 hours) and groups were counterbalanced for time of day effects. Mice were only administered drug or vehicle once. For the Geller-Seifter studies, male albino Sprague-Dawley rats (Charles River Labs, Wilmington, MA) weighing 250–300 g were kept on a restricted diet of Purina Lab Chow food pellets with water available at all times to maintain body weight at 85% of their free-feeding young adult levels. Rats were housed individually under a 12-h light-dark cycle with lights on from 7:00 a.m. to 7:00 p.m.

Anti-pentylenetetrazol test

CF/1 mice were pretreated with various doses of either 3α,5α-P or 3α,5β-P, 10 min prior to pentylenetetrazol (PTZ). The test compounds were administered intraperitoneally (IP) in a volume equivalent to 5 ml/kg. PTZ (85 mg/kg) was administered subcutaneously (SC) in a volume equivalent to 5 ml/kg. Mice were then placed in individual cages for observation of clonic seizures during a 30-min test period.

Roto-rod test

The roto-rod apparatus consisted of a constant rotating rod of 1 inch diameter, divided into four equivalent spaces. Photocells located below the rotating drum automatically measured the time spent on the drum. The rotating drum was set for six rotations per minute.

CF/1 mice were trained to stay on the rotating drum for a continuous 2 min prior to testing. 3α,5α-P and 3α,5β-P were administered IP (5 ml/kg) 10 min prior to testing. During testing, each animal was given three opportunities to stay on the rotating drum for a contiguous 60 s. Animals which failed to stay on the rotating drum for 60 s during all three trails were recorded as failing; animals that remained on the rotating drum for an uninterrupted 60 s during any of three trials were recorded as passing.

Light/dark transition test

The method used was a modification of methods previously described (Crawley and Goodwin 1980; Wieland et al. 1991; Young and Johnson 1991). The apparatus include two, two-compartment automated test chambers (Model RXYZCM16, Omnitech Electronics, Columbus, Ohio). The open compartment was connected to the enclosed compartment via a 7.5 × 7.5 cm passageway. The open compartment was brightly lit using a 200-W incandescent light bulb. The experimental room was kept dark. Interruptions of the infrared beams in either chamber were automatically recorded by being linked to a computer through a Digiscan Analyzer (Omnitech Electronics) and the data were analyzed using the Integrated Lab Animal Monitoring System (Omnitech Electronics).

NIH Swiss-Webster mice received vehicle or test drug IP; 10 min later they were placed in the center of the lit compartment. The number of transitions between the lit and dark chambers, total activity in the lit chamber and the time spent in the lit chamber were measured during a 5-min test period.

Elevated plus-maze

The method used was described previously (Lister 1987). The apparatus included two open arms perpendicular to two enclosed arms elevated 50 cm from the floor. Each arm was 50 cm long and the walls of the enclosed arms were 40 cm tall. The maze was made completely of black Plexiglas. Incandescent 200-W light bulbs were above each of the open arms to produce a strong contrast between the open arms and the enclosed arms.

Ten minutes after an IP injection, the NIH Swiss-Webster mice were placed in the center of the plus-maze facing an open arm. During the 5-min test period, the number of entries onto the open arms and the enclosed arms, and the time spent in the open arms and enclosed arms were measured. All four paws had to be within an arm for the dependent variable to be measured. Therefore, the time spent in the center of the maze is not counted, so the total time spent in the open arms and the enclosed arms may not equal 5 min.

Geller-Seifter test

The antianxiety (punishment-lessening) and response depressant effects of 3α,5β-P and 3α,5α-P were measured in rats by the conflict test of Geller and Seifter (1960). In this 63-min test, hungry rats perform a lever-press response to obtain a sweetened milk reward. The reinforcement schedule consists of 3-min punishment and 12-min non-punishment components, alternating approximately every 15 min. Rats were trained in test chambers (Coulbourn Instruments) with a lever mounted in one wall, a small dipper that

delivered the 0.1-ml milk reward (1 part Eagle condensed milk: 2 parts water), and a metal grid floor through which the foot-shock punishment was administered. A DEC PDP 11/73 minicomputer running SKED (State Systems) was used for programming and recording.

Rats initially learned to respond on a continuous reinforcement schedule and progressed rapidly to 30-s, 1-min, and 2-min variable interval (VI) schedules. On the continuous reinforcement schedule, rats received milk reward following every lever press; on the VI schedules, milk rewards were available at infrequent and variable intervals, eventually at an average of once every 2 min. Four 3-min "conflict" periods were then introduced on the unpunished VI baseline; the first started after 3 min of VI performance and the others were alternated between 12-min periods of VI responding. During conflict periods, which were signalled by the presentation of a light and a tone, the continuous reinforcement schedule was again in force and each lever press delivered both a milk reward and a brief (0.25 ms) foot-shock punishment. Shock intensity was 0.2 mA initially, and was increased daily in increments of 0.02 mA in order to gradually suppress lever pressing to five responses or fewer per conflict period. This training took 4–5 weeks, after which stable low rates of response were observed during conflict periods and stable high rates in the non-punishment periods. Drug-induced increases in the rate of punished responses were taken as an index of anti-anxiety activity, while decreases in the rate of unpunished responses were taken as an index of response depression or sedation.

Rats were treated (SC) with either 3α,5α-P or 3α,5β-P 30 min prior to behavioral tests. Similar volumes of vehicle were administered on the day before each drug test to measure baseline response rates.

Locomotor activity

Unhabituated CF/1 mice were placed in the center of a large Plexiglas box (42 × 42 × 30.5 cm) and the total distance traveled measured during a 10-min test period. The Digiscan Activity Monitor (Model RXYZCM16; Omnitech Electronics, Columbus, Ohio) included 16 photobeams that surround the box. The activity monitor was linked to a computer through a Digiscan Analyzer (Omnitech Electronics) and the data were analyzed using the Integrated Lab Animal Monitoring System (Omnitech Electronics). Mice received various doses of vehicle, 3α,5α-P or 3α,5β-P IP 10 min prior to being placed in the center of the activity boxes.

Drugs

The neuroactive steroids 3α,5α-P and 3α,5β-P were synthesized by CoCensys. Alprazolam and PTZ were purchased from Sigma (St. Louis, MO). 2-Hydroxypropyl-β-cyclodextrin (HPβCD) was purchased from Aldrich (Milwaukee, WI). The neuroactive steroids and alprazolam were solubilized in 20% HPβCD in saline and sonicated overnight. PTZ was dissolved in saline (0.9% NaCl).

Statistics

The anti-PTZ data were expressed as the percent number of animals protected against PTZ-induced convulsions. The dose at which 50% of the animals were protected (ED_{50}) was calculated using the method of Litchfield and Wilcoxon (1949). Individual comparisons between 3α,5α-P and 3α,5β-P were made using the Chi-square test at individual doses. The roto-rod results were analyzed in a similar fashion. The data were expressed as the percent number of animals failing the test. The dose at which 50% of the animals show toxicity (TD_{50}) was calculated using the method of Litchfield and Wilcoxon (1949). Individual comparisons were made using the Chi-square test. The protective index (PI) describes the relative safety margin of a drug based on its effects against PTZ and its effects on the roto-rod test; $PI = TD_{50}/ED_{50}$. The effects of each drug on locomotor activity were analyzed using a one-way ANOVA, followed by individual dose comparisons to vehicle control using Dunnett's *t*-test. The light/dark transition test and elevated plus-maze test results were displayed as a percent of control for comparison purposes. However, the data were analyzed using one-way ANOVAs, followed by Dunnett's *t*-test. Differences in punished and unpunished lever presses were calculated for each rat after drug and vehicle treatments; thus, each animal served as its own control. Statistical comparisons were made on the averaged individual difference scores following each drug dose and its preceding vehicle baseline score, using Student's *t*-test for paired comparisons.

Results

Anti-pentylenetetrazol test

The ability of 3α,5α-P and 3α,5β-P to block convulsant effects induced by PTZ is shown in Fig. 1a. Both neuroactive steroids produced overlapping dose-response curves with the ED_{50} values calculated to be 2.8 mg/kg for 3α,5α-P and 3.0 mg/kg for 3α,5β-P. There were no significant differences in responses to PTZ between 3α,5α-P and 3α,5β-P-treated animals at any dose tested.

Roto-rod test

3α,5α-P and 3α,5β-P exhibited dose-dependent ataxic effects as measured by the roto-rod test (Fig. 1b). The TD_{50} values were calculated to be 18.8 mg/kg for 3α,5α-P and 21.2 mg/kg for 3α,5β-P. There were no significant differences in roto-rod deficit between 3α,5α-P and 3α,5β-P at any dose tested.

The PI (TD_{50}/ED_{50}) values for 3α,5α-P and 3α,5β-P were 6.7 and 7.0, respectively. These results demonstrated that these two steroids produced similar profiles in the anti-PTZ and roto-rod tests.

Light/dark transition test

Figure 2 shows the effects of 3α,5α-P and 3α,5β-P in the light/dark transition test. 3α,5α-P and 3α,5β-P produce significant dose-response curves in relation to the number of transitions between the dark box and the light box [Fig. 2a; $F(4, 123) = 9.7$; $P = 0.0001$; $F(4, 138) = 5.54$; $P = 0.0004$, respectively]. Post-hoc comparisons showed that both compounds at 10 mg/kg ($P \leq 0.05$) and 20 mg/kg ($P \leq 0.01$) were significantly different from control (Dunnett's *t*-test). Individual comparisons between 3α,5α-P and 3α,5β-P showed that at 1.0 mg/kg, 3α,5β-P produced significantly ($P = 0.019$) greater increase in transitions (*t*-test) than the 5α isomer. Vehicle-treated animals produced an average 25.2 ± 2.7 crosses.

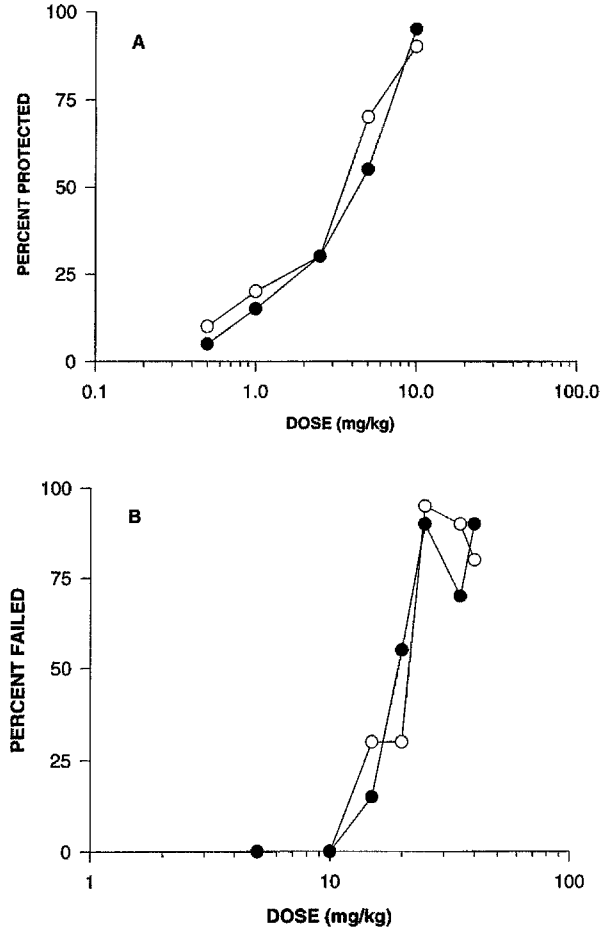

Fig. 1 The anti-PTZ (**A**) and roto-rod (**B**) effects of 3α,5α-P and 3α,5β-P. **A** Separate groups of mice were pretreated (IP) with increasing doses of either 3α,5α-P or 3α,5β-P 10 min prior to PTZ (85 mg/kg; SC). Mice were observed for clonic seizures for 30 min. The calculated ED_{50} values for 3α,5α-P was 2.8 mg/kg and 3.0 mg/kg for 3α,5α-P. There were no significant differences between the ability of 3α,5α-P and 3α,5β-P to antagonize PTZ-induced seizures. Each data point represents 20 animals. **B** Separate groups of mice were pretreated (IP) with increasing doses of either 3α,5α-P or 3α,5β-P 10 min prior to being tested on a roto-rod (6 rpm). The data were expressed as percent failing. The calculated TD_{50} for 3α,5α-P was 18.8 mg/kg and 21.2 mg/kg for 3α,5β-P. The TD_{50} values were not significantly different. Each data point represents 20 animals. O 3α,5α-P, • 3α,5β-P

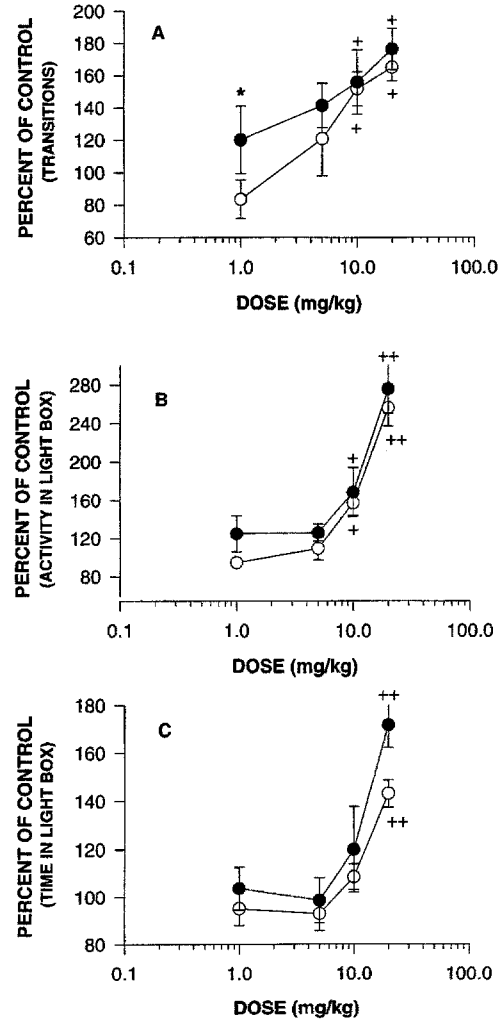

Fig. 2 The effect of 3α,5α-P and 3α,5β-P in the light/dark transition test. **A** The number of transitions between the light and dark boxes. Mice received either 3α,5α-P or 3α,5β-P IP 10 min prior to being placed on the lit side of a two-compartment box. The number of transitions were counted for 10 min. **B** The amount of activity in the lit box as defined as the number of photobeams broken. **C** The amount of time spent in the lit box during the 10-min test session. Each point represents the percent of control based on the mean of 24–27 animals per dose. $^{*}P \leq 0.05$, significantly different from 3α,5α-P. $^{+}P \leq 0.05$, $^{++}P \leq 0.01$; significantly different from control. • 3α,5α-P, O 3α,5β-P

Both 3α,5α-*P* and 3α,5β-*P* also produced significant increases in the percentage of activity in the light box [Fig. 2b; $F(4, 123) = 18.6$; $P = 0.0001$ and $F(4, 138) = 23.6$; $P = 0.0001$, respectively]. For both compounds, 10 and 20 mg/kg produced significant ($P \leq 0.01$) increases in activity as compared to control groups (Dunnett's *t*-test). However, there were no significant differences in this measurement between the two compounds at any dose tested. Vehicle-treated animals produced an average activity score of 728 ± 63.1.

3α,5α-P and 3α,5β-P significantly increased the time spent in the light box over control [Fig. 2c; $F(4, 123) = 6.185$; $P = 0.0001$ and $F(4, 138) = 14.39$; $P = 0.0001$, respectively]. For both compounds, 20 mg/kg produced significant ($P \leq 0.01$; Dunnett's *t*-test) increases in the amount of time in the light-compartment. There were no significant differences between 3α,5α-P and 3α,5β-P at any dose tested. The average time spent in the light-compartment for the vehicle-treated animals was 16.7 ± 10.9 s.

Elevated plus-maze

The effects of 3α,5α-P and 3α,5β-P on mice assessed by the elevated plus-maze test are shown in Fig. 3. 3α,5α-P and 3α,5β-P increased the proportion of entries into

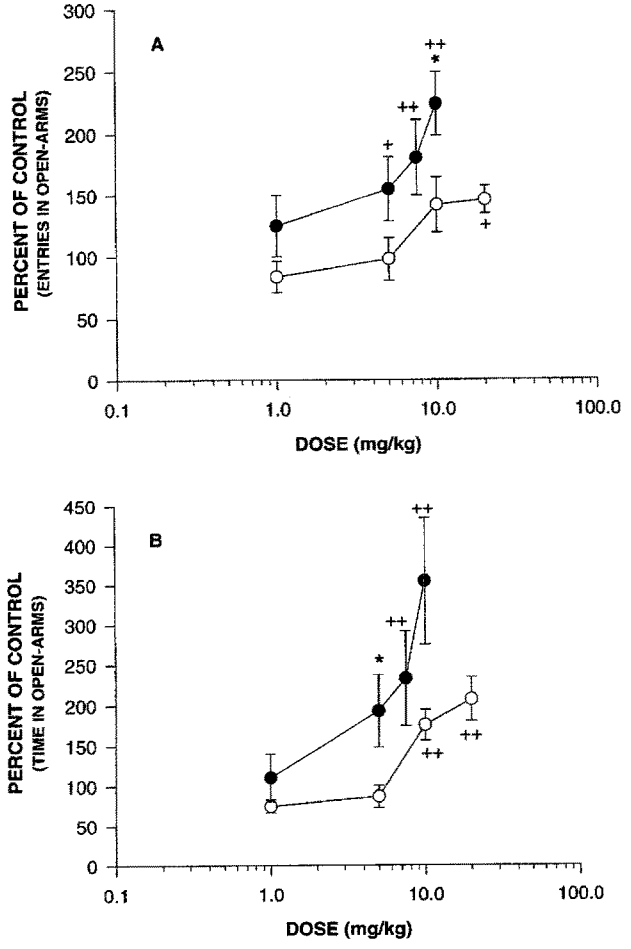

Fig. 3 The effects of 3α,5α-P and 3α,5β-P in the elevated plus-maze test. **A** Percent number of entries into the open-arms. Mice received (IP) either 3α,5α-P or 3α,5β-P 10 min prior to being placed in the center of the plus-maze apparatus. The number of entries were counted for 5 min. **B** Percent time spent in the open-arms. Each point represents the percent of control based on the mean of 24–27 animals per dose. $^{*}P \leq 0.05$; significantly different from 3α,5α-P. $^{+}P \leq 0.05$, $^{++}P \leq 0.01$; significantly different from control. ○ 3α,5α-P, ● 3α,5β-P

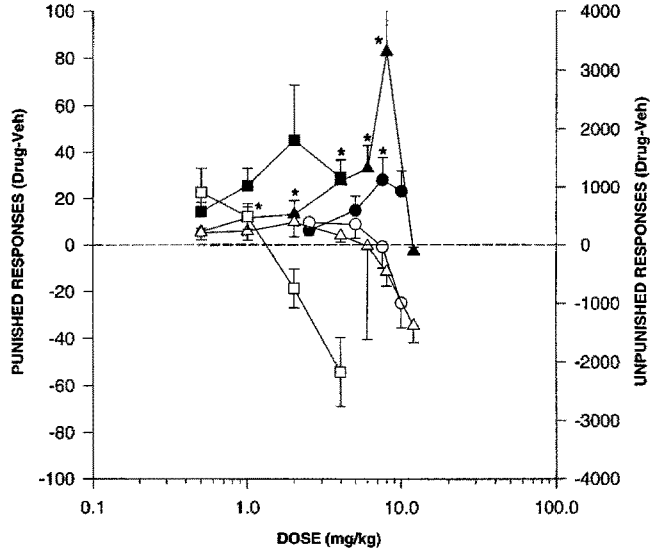

Fig. 4 Dose-response curves for 3α,5α-P and 3α,5β-P in the Geller-Seifter conflict test. Groups of rats with stable baselines of punished and unpunished behavior in this test received different doses of test compound no more frequently than once per week 30 min before the onset of testing. Drug effects were expressed as differences from response scores obtained on the previous day after pretreatment with the vehicle. Significant increases in punished behavior are indicative of anti-anxiety activity were produced by both compounds. $^{*}P \leq 0.05$; significantly different from control. ● Pun-3α,5α-P, ○ Unpun-3α,5α-P, ▲ Pun-3α,5α-P, △ Unpun-3α,5β-P, ■ Pun-Alprazolam, □ Unpun-Alprazolam

the open-arms across doses [Fig. 3a; $F(4, 144) = 6.722$; $P = 0.0001$ and $F(4, 144) = 13.8$; $P = 0.001$, respectively]. 3α,5β-P significantly increased the proportion of entries over control at 5 mg/kg ($P \leq 0.05$), 7.5 mg/kg ($P \leq 0.01$) and 10 mg/kg ($P \leq 0.01$) (Dunnett's *t*-test). 3α,5α-P was only significantly different from control at 20 mg/kg ($P \leq 0.05$) Dunnett's *t*-test). In addition, at 10 mg/kg 3α,5β-P produced a significantly greater effect than 3α,5α-P ($P \leq 0.02$; Mann-Whitney *U*-Test). Though not statistically significant, all doses of 3α,5β-P produced an apparently greater proportion of entries into the open-arms as compared to 3α,5α-P.

In addition, 3α,5α-P [$F(4, 144) = 16.0$; $P = 0.001$] and 3α,5β-P [$F(4, 144) = 22.4$; $P = 0.001$] produced dose-dependent increases in the time spent in the open-arms (Fig. 3b). 3α,5β-P significantly increased the proportion of time spent in the open-arms over control at 7.5 mg/kg and 10 mg/kg ($P \leq 0.01$; Dunnett's *t*-test). Similarly, 3α,5α-P produced significant effects over control at 10 mg/kg and 20 mg/kg ($P \leq 0.01$). Although 3α,5β-P produced apparently greater effects than 3α,5α-P at all doses, significance was reached only at 5 mg/kg ($P \leq 0.025$; Mann-Whitney *U*-test).

Geller-Seifter

The effects of 3α,5β-P and 3α,5α-P in the conflict test are summarized in Fig. 4. Both compounds produced significant increases in the rate of punished responses, suggesting that both would be active as anti-anxiety agents. The peak effect of 3α,5β-P was observed at 8 mg/kg, SC and that of 3α,5α-P at 7.5 mg/kg, SC 3α,5β-P: $t = 3.72$, $P \leq 0.02$; 3α,5α-P: $t = 2.91$, $P \leq 0.01$). 3α,5β-P also produced significant increases in punished responding at 1, 2, 4 and 6 mg/kg. Thus, 3α,5β-P was more potent than 3α,5α-P which only produced significant effects at 7.5 mg/kg. A decrease in responding was observed at the highest dose tested for each compound. Alprazolam produced peak effects at 2 mg/kg. 3α,5β-P produced a maximum number of punished responding 2-fold greater than that of alprazolam. Alprazolam appears to be more sedating than either of the neuroactive steroids.

Locomotor activity

The effects of 3α,5α-P and 3α,5β-P on unhabituated locomotor activity is shown in Fig. 5. Two-factor analysis of variance showed a significant main drug effect [$F(1, 119) = 16.12$; $P \leq 0.0001$], significant main dose effect [$F(6, 119) = 25.4$; $P \leq 0.0001$], but no significant drug × dose interaction. Further analysis of the data showed that 3α,5β-P produced a significant increase in locomotor activity at 10 mg/kg. 3α,5α-P produced significant effects at 5, 10 and 20 mg/kg. Both drugs significantly reduced locomotor activity at 40 mg/kg. However, there were no significant differences between the two steroids, as seen by a lack of a drug × dose interaction.

Discussion

Benzodiazepines and imidazopyridines produce their pharmacological actions through $GABA_A$/benzodiazepine receptor complexes. These compounds act as positive allosteric modulators of GABA action by their interactions with the benzodiazepine binding site. Because of this shared mechanism of action, these compounds all elicit anticonvulsant, anxiolytic, sedative/hypnotic, and muscle relaxing properties. However, the relative efficacies of these compounds for these pharmacological properties are different. Thus, the anxiolytic property of diazepam overlaps with its sedative activity, triazolopyridazine CL 218872 has anxiolytic

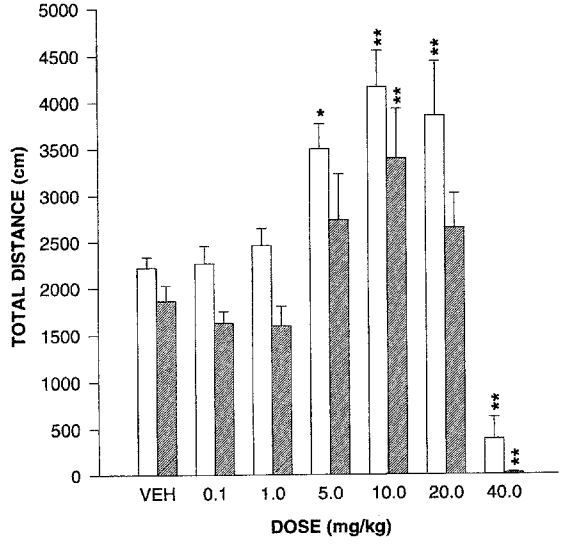

Fig. 5 The effects of 3α,5α-P and 3α,5β-P on locomotor activity. Separate groups of mice received either neuroactive steroid IP 10 min prior to being placed in the center of an automated locomotor chamber. 3α,5α-P significantly increased locomotor activity at 5, 10 and 20 mg/kg, while 3α,5β-P produced a significant increase at 20 mg/kg compared to vehicle controls. However, both neuroactive steroids produced significant sedation at 40 mg/kg. $^*P \leq 0.05$; $^{**}P \leq 0.01$ compared with vehicle control. □ 3α,5α-P, ▨ 3α,5β-P

effects but is only weakly sedative, whereas zolpidem [N, N, 6-trimethyl-2-(4-methylphenyl)imidazol (1,2-a) pyrindine-3-acetamide hemitartrate] is a potent hypnotic but weak anticonvulsant and anxiolytic. This differential activity may be partially contributed by the subtype selectivity of these compounds for the benzodiazepine receptor (Zivkovic et al. 1988). Heterogeneity of the GABA/benzodiapine receptors has been demonstrated pharmacologically and revealed by molecular cloning (Burt and Kamatchi 1991; Vicini 1991). However, a direct correlation of subtype selectivity with the pharmacological properties of benzodiapines has not been delineated.

Previous studies with neuroactive steroids have not shown anxiolytic or sedative effects after systemic administration of 3α,5α-P (Mendelson et al. 1987). However, increased locomotor activity and exploratory activity in the elevated plus-maze have been demonstrated for 3α,5α-P and 3α,5β-P after intracerebroventricular administration (Bitran et al. 1991). This study further suggested that 3α,5α-P was more potent than 3α,5β-P, but less efficacious (Bitran et al. 1991). Because both 3α,5α-P and 3α,5β-P are insoluble in a variety of vehicles including those used in the systemic studies, it is possible that the inactivity observed may be due to the low bioavailibility of the compounds. In the current study, we used 20% HPβCD as a vehicle which solubilizes both compounds to the extent of the dose range tested. The present study systematically examined anticonvulsant, anxiolytic, locomotor activity effects elicited by 3α,5α-P and 3α,5β-P by IP and SC administration. We found that dose-response curves of 3α,5α-P and 3α,5β-P in blocking PTZ-induced convulsions are superimposable, suggesting that they have similar anticonvulsant activities. However, under exactly the same experimental conditions, the 5β isomer consistently showed more robust anxiolytic properties. In that, the 5β isomer was more efficacious than the 5α isomer in light/dark transition and elevated plus-maze test, whereas it was more potent and efficacious than the 5α isomer in the Geller-Seifter test. The differential anxiolytic properties observed here when compounds were administered parentally are consistent with the results obtained from the ICV study (Bitran et al. 1991).

Neuroactive steroids, like benzodiapines, produced their action through the $GABA_A$ receptor complex but they act on a binding site which is distinct from the benzodiazepine binding site (Gee 1988). Current understanding of this neuroactive steroid binding site is limited. However, it is clear that unlike benzodiazepines, the γ-subunit of the $GABA_A$ receptor is not required for reconstituting the neuroactive steroid activity. Subtype selectivity by neuroactive steroid ligands for the $GABA_A$ receptor as well as their differential pharmacological behavior have not been illustrated. The present study demonstrates that the small difference in the orientation of the steroid A-ring (cis versus

transfusion to the B-ring) of the two isomers showed some selective differences, albeit small, in the in vivo pharmacology. Thus, if the notion is correct that receptor subtype selectivity is responsible for the various pharmacological consequences of ligand interaction with the $GABA_A$ receptor, including unwarranted side effects, further exploration with synthethic neuroactive steroids may delineate such a relationship. This latter knowledge would undoubtedly provide an insight into the design of drugs for a specific indication with better therapeutic index.

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