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Antineophobic Effect of the Neuroactive Steroid 3α-Hydroxy-5β-pregnan-20-one in Male Rats

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HIGGS, S. AND S. J. COOPER. *Antineophobic effect of the neuroactive steroid 3α-hydroxy-5β-pregnan-20-one in male rats.* PHARMACOL BIOCHEM BEHAV **60**(1) 125–131, 1998.—The neuroactive steroid 3α-hydroxy-5β-pregnan-20-one (pregnanolone) and benzodiazepine receptor (BZR) agonists share sedative, anxiolytic, and anticonvulsant properties. Recent evidence suggests that like BZR agonists, pregnanolone may also modulate feeding responses. The present experiments examined the behavioral mechanisms responsible for any hyperphagic effect of pregnanolone. The effect of pregnanolone (1–10 mg/kg IP) on the intake and microstructure of licking for two sucrose solutions (1 and 3%) in well familiarized nondeprived male rats under either light or dark conditions was examined. Pregnanolone had no effect on either intake or the duration or number of bouts of licking in these experiments, although in all cases the intrabout lick rate was significantly reduced at the highest dose. Pregnanolone (1–10 mg/kg) also failed to increase intake of a sweet wet mash in familiarized nondeprived male rats. However, in a food choice test where both novel and familiar food items were available, pregnanolone (1–3 mg/kg) significantly increased the time spent eating the novel food. These results suggest that unlike BZR agonists, which enhance feeding responses directly, pregnanolone may facilitate feeding secondarily via an attenuation of anxiety. © 1998 Elsevier Science Inc.

Neuroactive steroid Pregnanolone Food intake Novelty Antineophobia

IT is well known that some pregnane steroids can rapidly alter CNS excitability via nongenomic mechanisms [see (22,26)]. The term neuroactive steroid has been coined to describe these compounds that can be synthesized in the brain from cholesterol (1). Neuroactive steroids have been shown to allosterically modulate GABA transmission via action at a unique binding site on the $GABA_A$ receptor complex (17). Molecular and biochemical techniques have demonstrated that the mechanism by which neuroactive steroids affect GABAergic transmission differs from that of both benzodiazepines and barbiturates (18,23,25,27).

Positive modulators of $GABA_A$ function, such as the 3α-hydroxylated pregnane steroid 3α-hydroxy-5β-pregnan-20-one (pregnanolone), are known to elicit a wide range of behavioral effects including anticonvulsant, anxiolytic, and sedative/hypnotic actions (2,14,26). For example, pregnanolone has been shown to be anxiolytic in the elevated plus maze (5), the light/dark transition test (31), and the Geller-Seifter test (31). Wieland and colleagues (31) have also shown that pregnanolone blocks pentylenetetrazol-induced seizures and induces rotorod deficits in mice.

This profile is strongly reminiscent of the behavioral effects of benzodiazepine receptor (BZR) agonists. Because BZR agonists also produce increases in food intake, it might be predicted that neuroactive steroids should induce a hyperphagic response. In confirmation of this, it has recently been shown by Chen et al. (6) that the 3α-hydroxylated pregnane steroids pregnanolone, allopregnanolone, and alphaxalone stimulated a significant increase in consumption of sweetened pellets in nondeprived male rats. However, this interesting result refers only to the amount of food consumed and, therefore, the behavioral mechanisms involved in the putative hyperphagic effects of these compounds remain undetermined.

There is compelling evidence that BZR agonists induce increases in food intake by direct means and not as a secondary consequence of their anxiolytic properties (7). In particular, it has been proposed that BZR agonists achieve their appetite-stimulant effects by enhancing palatability (3,10). Evidence in support of this comes from the positive effects of BZR agonists in various tests of palatability, including taste reactivity tests (4,19,30), taste preference tests (9,13), and the sham-feeding paradigm (12). The effect of the potent BZR agonist

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midazolam on the microstructure of licking behavior in the rat is also consistent with a palatability interpretation of BZR agonist-induced hyperphagia. Davis and colleagues (15,16) have shown that the duration of bouts of licking are positively related to sugar concentration and so provide a good estimate of the palatability of ingested fluids. The recent finding that midazolam increases the duration of bouts of licking in brief access tests is fully consistent with the hypothesis that BZR agonists act directly to enhance the palatability of ingested fluids (20).

The aim of the present work was to follow the benzodiazepine example and employ a microstructural approach to characterize the behavioral changes associated with pregnanolone-induced hyperphagia. If this neuroactive steroid has a direct effect on feeding, comparable to that of BZR agonists, then we should expect it to increase the licking for a palatable sweet sucrose solution and to increase the average duration of bouts of licking. In the first four experiments, methods were used that have been successful in identifying BZR-induced changes in ingestive responding. The final experiment, however, employed a food preference test (8) to evaluate the possibility that pregnanolone affects ingestive behavior only through a reduction in food neophobia.

EXPERIMENTS 1–4

Method

Animals. Thirty-seven nondeprived adult male hooded Lister rats (Charles River, UK), weighing 300–400 g at the beginning of training, were used. They were housed in pairs in plastic cages in a room with a constant temperature of 21 ± 2°C and were maintained under a 12 L:12 D cycle (lights on at 0600 h). Rats were allowed ad lib access to food pellets [SDS RM1 (E), Cambridge, UK] and water, except during experimentation. The experiments were carried out in accordance with the terms of the Animals (Experimental Procedures) Act, 1986 under licence from the UK Home Office.

Drugs. The 3α-hydroxylated pregnane steroid pregnanolone was prepared for injection either by ultrasonic dispersion in distilled water to which Tween 80 (BDH Chemicals Ltd, Poole, UK) had been added (Experiments 1, 2, and 3), or by dissolving in a 20% solution of β-cyclodextrin (Sigma, Poole, UK) followed by sonication for 30 min (Experiment 4). The doses were 1, 3, and 10 mg/kg or the appropriate vehicle. This dose range has been previously shown to increase intake of sweetened chow in nondeprived male rats (6). Pregnanolone was administered IP 30 min before testing in a volume of 1 ml/kg.

Test meal. Sucrose: rats had access to either a 3% (Experiments 1 and 2) or a 1% (Experiment 4) weight/volume sucrose solution (granulated cane sugar, Tate and Lyle, UK) which was made up freshly each day using tap water.

Mash: in Experiment 3, rats had access to a sweetened mash meal that was made up daily according to the following formula: 50 ml sweetened condensed milk, 200 ml ground maintenance diet [SDS RM1(E), Cambridge, UK], and 100 ml tap water. The constituents were mixed to produce a soft mash. This recipe has previously been shown to be readily consumed by nondeprived rats (11).

Training and testing. Sucrose: familiarization with the sucrose solutions occurred until steady baseline levels of intake were observed and consumption did not differ by more than 2 ml on 3 consecutive days (approximately 10 days). This involved transferring the rats from their home cages to testing cages in which they had access to a single drinking spout attached to a 50 ml graduated cylinder. Two days prior to testing each animal received a sham injection of distilled water to familiarize it with the injection procedure. On test days, rats received an IP injection of pregnanolone (1, 3, or 10 mg/kg) or vehicle and were then returned to the home cage for 30 min before being transferred to the test cage. Testing was carried out either in the light phase between 1400 and 1600 h (Experiments 1 and 4) or in the dark phase 1 h after lights out (between 1900 and 2100 h) under red light (Experiment 2).

Mash: in Experiment 3, the rats were first familiarized with the test meal and experimental procedure until a steady baseline intake of mash was observed (10 days). This involved each rat having 30 min access to 50 g portions of the diet placed in a clear plastic Petri dish inside an individual plastic cage. The consumption of the sweetened mash was then measured to the nearest 0.1 g with corrections made for any spillage. The test cage was identical to the home cage but did not contain sawdust, food, or water. Two days prior to testing, each animal received a sham injection of distilled water. On test days, rats received an IP injection of pregnanolone (1, 3, or 10 mg/kg) or vehicle and were then returned to the home cage for 30 min before being transferred to the test cage. All testing was carried out during the light phase between 1000 and 1200 h to ensure relatively low baseline levels of consumption.

Data recording. The microstructural data were collected using a Contact 108 lick analysis system (Dilog Instruments, Tallahassee, FL). This consisted of a lick block that was attached to the front of a plastic cage (identical to the home cage), and which allowed a stainless steel drinking spout to be positioned 5.5 cm from the floor of the cage. The lick block was connected to an amplifier that passed less than 60 nA through the rat every time tongue contact with the tube was made. This current was fed to a computer (Standard PC, Opus Technology, Surrey, UK), which stored the time of each tongue contact to the nearest ms. Sucrose intake was also recorded volumetrically to the nearest milliliter after a 30-min test period.

The lick data were grouped into bouts using software written by Dave Barton (University of Sunderland, UK). The cutoff point for defining a bout was set as an upper interlick interval (ILI) of 400 ms. The software then calculated the number of bouts and the duration of each bout within a presentation. The mean bout duration was calculated by summing over the presentation and taking an average value for each rat. Another parameter examined was the intrabout lick rate. This was calculated as the number of licks in a bout minus 1, divided by the total duration of the bout.

Design and analysis. A repeated-measures design was used throughout in which each animal served as its own control. The order of injections was counterbalanced and 48 h elapsed between successive injections to allow for dispersal of the drug. The data were analyzed using a one-way analysis of variance (ANOVA) for repeated-measures. Statistical tests were performed using Statview SE+graphics (Abacus Concepts Inc., Berkeley, CA) and a result was considered statistically significant if $p < 0.05$.

Results and Discussion

Experiment 1. The effect of pregnanolone on consumption of a 3% sucrose solution in nondeprived rats tested in the light phase. Intake: the effect of pregnanolone on total intake of 3% sucrose over the 30-min test session is shown in Table 1. A one-way repeated-measures ANOVA showed that the baseline intake of 18 ml was not significantly affected as a result of drug treatment, $F(3, 27) = 0.09$, NS.

TABLE 1

THE EFFECT OF PREGNANOLONE (1–10 mg/kg) ON INGESTION OF A 3% SUCROSE SOLUTION IN NONDEPRIVED RATS TESTED IN THE LIGHT PORTION OF THE LIGHT:DARK CYCLE

	Pregnanolone (mg/kg)			
Measure	Vehicle	1 mg/kg	3 mg/kg	10 mg/kg
Intake (ml)	17.7 ± 1.5	17.8 ± 1.6	18.4 ± 1.5	17.8 ± 1.7
Bout duration (s)	3.7 ± 0.3	4.0 ± 0.3	4.1 ± 0.4	4.1 ± 0.5
Number of bouts	117.4 ± 10.9	117.3 ± 16.7	111.5 ± 13.5	119.3 ± 16.6
Intrabout lick rate (l/s)	6.9 ± 0.1	6.7 ± 0.1	6.8 ± 0.09	6.6 ± 0.09*

Results are shown as mean ± SEM after 30 min. $n = 10$ rats per condition. Asterisk indicates reliably different from vehicle condition $^*p < 0.01$ (Dunnett's *t*-test).

Microstructural analysis: mean bout duration—a one-way repeated-measures ANOVA revealed that there was no significant effect of pregnanolone on the mean bout duration for sucrose drinking over the 30-min test session, $F(3, 27) = 0.67$, NS (Table 1).

Number of bouts—as shown in Table 1, the number of bouts did not vary significantly as a result of treatment with pregnanolone, $F(3, 27) = 0.11$, NS.

Intrabout lick rate—the effect of pregnanolone on the intrabout lick rate for 3% sucrose is shown in Table 1. There was a reduction in the intrabout lick rate following pregnanolone treatment, $F(3, 27) = 3.94$, $p < 0.05$, and a post hoc Dunnett's *t*-test revealed that this was significant at the 10 mg/kg dose of pregnanolone ($p < 0.01$).

Pregnanolone had no effect on either the intake or bout structure of rats drinking a 3% sucrose solution. These results are not consistent with the findings of Chen et al. (6), who demonstrated that pregnanolone could induce a hyperphagia in nondeprived male rats. One difference between the method used in Experiment 1 and that of Chen and colleagues was the time of testing. These authors tested their rats in the dark portion of the light cycle, whereas in Experiment 1, the rats were tested in the light portion of the cycle. The aim of Experiment 2 was to examine if an increase in sucrose consumption following pregnanolone administration could be demonstrated when testing took place during the dark portion of the light cycle.

Experiment 2. The effect of pregnanolone on consumption of a 3% sucrose solution in nondeprived rats tested in the dark phase. Intake—as shown in Table 2, there was no effect of pregnanolone on consumption of a 3% sucrose solution after 30 min when testing in the dark phase of the light:dark cycle. The average baseline level of intake of 20 ml was not significantly altered following administration of pregnanolone, $F(3, 27) = 0.54$, NS.

Microstructural analysis: mean bout duration—the effect of pregnanolone on mean bout duration over 30 min is shown in Table 2. There was no significant effect of drug treatment on this parameter, $F(3, 27) = 0.65$, NS.

Number of bouts—a repeated-measures ANOVA showed that pregnanolone did not significantly affect the number of bouts for 3% sucrose over the 30-min test period, $F(3, 27) = 0.9$, NS. The results are summarized in Table 2.

Intrabout lick rate—administration of pregnanolone did significantly alter the intrabout lick rate for 3% sucrose. As shown in Table 2, the intrabout lick rate was reduced following pregnanolone treatment, $F(3, 27) = 3.63$, $p < 0.05$. A post hoc test revealed that the 10 mg/kg dose of pregnanolone was effective in reducing the intrabout lick rate ($p < 0.05$)

The failure of pregnanolone to increase sucrose consumption or alter the duration or number of bouts of licking in Experiment 2, when testing was done in the dark portion of light:dark cycle, suggests that differences in the time of testing cannot account for the failure to replicate the results of Chen et al. (6). An additional difference between the method employed by Chen and colleagues and the method used in the current experiments is that they gave their rats a test meal of solid chow sweetened with sucrose, whereas the test meal in

TABLE 2

THE EFFECT OF PREGNANOLONE (1–10 mg/kg) ON INGESTION OF A 3% SUCROSE SOLUTION IN NONDEPRIVED RATS TESTED IN THE DARK PORTION OF THE LIGHT:DARK CYCLE

	Pregnanolone (mg/kg)			
Measure	Vehicle	1 mg/kg	3 mg/kg	10 mg/kg
Intake (ml)	20.4 ± 1.6	19.3 ± 1.5	20.6 ± 1.5	19.4 ± 1.7
Bout duration (s)	3.4 ± 0.2	3.3 ± 0.3	3.3 ± 0.3	3.5 ± 0.3
Number of bouts	114.2 ± 7.6	105.8 ± 8.6	117.0 ± 11.8	101.1 ± 11.3
Intrabout lick rate (l/s)	6.9 ± 0.1	6.9 ± 0.1	6.8 ± 0.1	6.6 ± 0.1*

Results are shown as mean ± SEM after 30 min. $n = 10$ rats per condition. Asterisk indicates reliably different from vehicle condition $^*p < 0.05$ (Dunnett's *t*-test).

TABLE 3

THE EFFECT OF PREGNANOLONE (1–10 mg/kg) ON INGESTION OF A SWEET WET MASH IN NONDEPRIVED RATS

	Pregnanolone (mg/kg)			
Measure	Vehicle	1 mg/kg	3 mg/kg	10 mg/kg
Intake (g)	8.5 ± 0.6	7.4 ± 0.8	7.4 ± 0.9	8.4 ± 0.6

Results are shown as mean intake (g) ± SEM after 30 min. $n = 10$ rats per condition.

the present experiments was a sucrose solution. The aim of Experiment 3 was to examine whether the lack of hyperphagic effect of pregnanolone observed in Experiments 1 and 2 was due to the specific nature of the test meal. Therefore, instead of having access to a 3% sucrose solution, rats had access to a sweetened mash that was more similar to the test meal used by Chen et al.

Experiment 3. The effect of pregnanolone on consumption of a sweet wet mash in nondeprived rats. Intake—the effect of pregnanolone on intake of the sweetened wet mash in the 30-min test session is shown in Table 3. A repeated-measures ANOVA revealed that pregnanolone did not significantly alter the average baseline consumption under the vehicle condition of 8.5 g, $F(3, 27) = 1.61$, NS.

An alternative explanation for the failure to replicate the results of Chen et al. (6) in the preceding experiments could be the difference in the drug vehicle used. In Experiments 1, 2, and 3 the vehicle was distilled water to which Tween 80 had been added, whereas Chen et al. (6) used a solution of β-cyclodextrin. It is unlikely like this difference could account for the discrepancy between the results because the effect of pregnanolone on the intrabout lick rate in the present experiments suggests that it was having a pharmacological effect using the Tween 80 vehicle (despite the fact that no specific alteration in bout structure could be demonstrated). In addition, behavioral effects of pregnanolone and allopregnanolone have been observed previously using Tween 80 as a dispersing agent (5,21). Nevertheless, the aim of Experiment 4 was to check this possibility by using β-cyclodextrin as the drug vehicle. An additional manipulation was to use a 1% sucrose solution instead of a 3% solution to determine if the lack of hyperphagic effect of pregnanolone observed in Experiments 1–3 was due to a ceiling effect as a result of fairly high baseline levels of consumption.

Experiment 4. The effect of pregnanolone on consumption of a 1% sucrose solution in nondeprived rats. Intake—Table 4 shows the effect of pregnanolone on total intake of a 1% sucrose solution over the test session. The relatively low level of intake under the vehicle condition of 9 ml was not significantly affected by drug treatment, $F(3, 18) = 1.47$, NS.

Microstructural analysis. Mean bout duration—a repeated-measures ANOVA showed that there was no significant effect of pregnanolone administration on mean bout duration over the 30-min session for 1% sucrose, $F(3, 18) = 2.1$, NS (Table 4).

Number of bouts—Table 4 shows that pregnanolone did not significantly affect the number of bouts in the test session for 1% sucrose, $F(3, 18) = 1.14$, NS.

Intrabout lick rate—the effect of pregnanolone on the intrabout lick rate for 1% sucrose is shown in Table 4. Pregnanolone significantly reduced the average intrabout lick rate, $F(3, 18) = 9.34$, $p < 0.001$. A post hoc test showed that the 10 mg/kg dose of pregnanolone was effective in reducing the intrabout lick rate ($p < 0.01$).

Experiments 1–4 failed to demonstrate any effect of pregnanolone on total intake or bout structure under a variety of testing conditions. However, one common factor in all of the above experiments was that the rats had been well familiarized with the test procedure. The procedure used by Chen et al. (6), who were able to demonstrate an increase in food intake following administration of pregnanolone, involved only 1 day of familiarization. This contrasts with the several days of training involved in Experiments 1–4. Therefore, it is possible that the hyperphagia observed by Chen and colleagues occurred as a result of pregnanolone reducing the reluctance to eat in a relatively novel situation. The aim of Experiment 5 was to test this possibility using a short-duration food preference test (requiring no training), in which rats are given a simultaneous choice between a novel and familiar food. This test allows a distinction to be drawn between a reduction in anxiety (which is indicated by an increase in the choice of the novel food) and an increase in appetite (which is indicated by an increase in the choice of the familiar food).

EXPERIMENT 5

Method

Animals. Thirty-six nondeprived adult male hooded Lister rats (Charles River, UK) weighing 300–400 g at the beginning of training were used. They were housed under the conditions described in Experiments 1–4.

TABLE 4

THE EFFECT OF PREGNANOLONE (1–10 mg/kg) ON INGESTION OF A 1% SUCROSE SOLUTION IN NONDEPRIVED RATS

	Pregnanolone (mg/kg)			
Measure	Vehicle	1 mg/kg	3 mg/kg	10 mg/kg
Intake (ml)	9.3 ± 1.5	8.4 ± 1.0	8.0 ± 1.3	6.1 ± 1.3
Bout duration (s)	2.6 ± 0.2	2.6 ± 0.3	2.4 ± 0.2	2.2 ± 0.2
Number of bouts	89.4 ± 22.7	77.4 ± 11.9	83.7 ± 15.3	65.3 ± 9.6
Intrabout lick rate (l/s)	6.8 ± 0.06	6.8 ± 0.09	6.7 ± 0.08	6.3 ± 01*

Results are shown as mean ± SEM after 30 min. $n = 7$ rats per condition. Asterisk indicates reliably different from vehicle condition $^*p < 0.01$ (Dunnett's *t*-test).

Drugs. Pregnanolone was dissolved in a 20% β-cyclodextrin solution (Sigma, Poole, UK) and sonicated for 30 min. The doses were 1 and 3 mg/kg or vehicle. Pregnanolone was administered IP 30 min before testing in a volume of 1 ml/kg.

Test meal. Rats had access to two types of food that were first, a familiar food item that was standard chow pellets (SDS RM1 (E), Cambridge, UK), and second, a novel food item that was either cheese biscuits (Ritz Crackers, Jacobs, Reading, UK) or ginger biscuits (Ginger Snap, Sainsburys, London, UK). All foods were prepared in pieces of comparable size and were placed in a shallow pile in a clear plastic Petri dish. There was one type of food per dish.

Testing. Testing took place in a perspex chamber that was approximately 16 cm wide, 30 cm long, and 20 cm tall. A perspex lid with four small holes in the top secured the chamber. The floor was made of a grid of stainless steel rods spaced 1 cm apart. One day prior to testing animals were familiarized with the testing environment by being placed in the test cage for 10 min with no food available. A sham injection of distilled water was also given. For the food preference test, each animal was deprived of food from 1700 h on the day prior to testing. Each animal was then tested individually the next day and was placed for 10 min in the test cage where they had access to the test foods. Half of the animals were given a choice between familiar chow and ginger biscuits and half of the animals had a choice between chow and Ritz biscuits. Two types of novel food were used to control for any effect being due to the specific properties of stimulus rather than being due to novelty. The time spent eating each food was recorded (s). Eating time was only recorded when the animals took food into the mouth and chewed; time spent sniffing or holding the food without eating was not recorded. After each trial a new food container was placed in the cage and any spillage was removed. Rats were randomly assigned to one of three groups: vehicle (20% β-cyclodextrin), 1 mg/kg, or 3 mg/kg pregnanolone.

Data analysis. The data were first log transformed due to the lack of normal distribution of the data. A three-way ANOVA was then performed on the log-transformed eating duration times with drug dose and biscuit type (ginger vs. cheese) as between subject factors and familiarity of the food items (familiar chow vs. novel biscuit) as a within-subjects factor.

RESULTS

A three-way ANOVA revealed that there was a main effect of drug treatment on eating duration, $F(2, 32) = 9.5$, $p < 0.001$. Pregnanolone significantly increased the total duration of eating during the 10 min session. Figure 1 shows that the increase in duration of eating brought about by pregnanolone could be accounted for solely in terms of an increase in the time spent eating the novel food: the drug had no effect on consumption of the familiar chow. This was confirmed by the significant interaction between familiarity and drug treatment, $F(1, 32) = 6.9$, $p < 0.01$. There was no interaction between drug treatment and biscuit type, $F(1, 32) = 1.7$, NS, indicating that the effect of pregnanolone was the same regardless of whether the novel food was ginger or cheese biscuits. There was a significant effect of familiarity, $F(1, 32) = 48.65$, $p < 0.001$, although no interaction between familiarity and biscuit type, $F(1, 32) = 1.7$, NS. The rats spent significantly less time eating the novel food under the vehicle condition (ginger or cheese biscuits), and this confirms that there was a neophobic reaction to the biscuits.

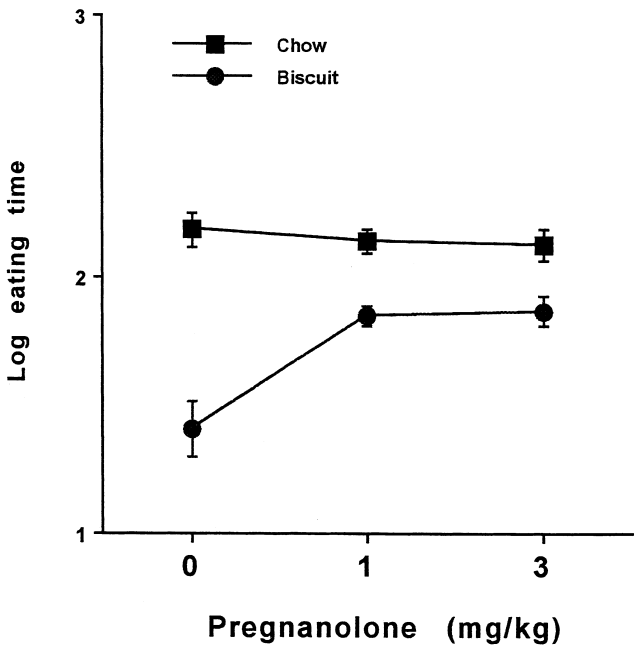

FIG 1. The effect of pregnanolone (1–3 mg/kg) on eating time scores (log-transformed) in a 10-min preference test according to food type consumed. Each point represents the mean ± SEM for 12 animals.

GENERAL DISCUSSION

The aim of the present studies was to examine the behavioral mechanisms underlying the effect of the neuroactive steroid pregnanolone on food intake in male rats. Experiments 1–4 failed to demonstrate any hyperphagic effect of this compound under a variety of testing conditions in well-trained rats. Only in Experiment 5, under conditions of novelty, was a stimulatory effect of pregnanolone on ingestion demonstrated. The data do not support the conclusion that pregnanolone directly enhances appetite but rather point to an antineophobic effect of pregnanolone.

In Experiments 1, 2, and 4, a microstructural approach to the study of ingestive behavior was adopted. No effect of pregnanolone on total intake of either a 3% (Experiments 1 and 2) or a 1% sucrose solution (Experiment 4) was observed. This was regardless of whether testing was carried out in the light (Experiment 1) or dark phase (Experiment 2) of the light cycle, or whether the vehicle used was distilled water plus Tween 80 (Experiments 1 and 2), or a 20% solution of β-cyclodextrin (Experiment 4). Pregnanolone also had no effect on the mean duration of bouts of licking in these experiments. Because mean bout duration has been shown to vary monotonically with increasing sugar concentration (15,16), this suggests that pregnanolone was not affecting palatability responses.

It is unlikely that the lack of effect of pregnanolone on sucrose consumption can be explained by ceiling effects because no increase in intake was observed even when the baseline intake was relatively low at 9 ml (Experiment 4). The failure of pregnanolone to increase sucrose intake cannot be explained by circadian effects either, as pregnanolone failed to affect sucrose consumption when testing was done in either the light or dark portions of the light:dark cycle. Finally, it is also unlikely that the lack of effect of pregnanolone on sucrose drink-

ing can be explained by a general lack of pharmacological activity of the drug because the results obtained did not depend upon the type of vehicle used, and importantly, pregnanolone did significantly affect one of the microstructural variables examined: the rate of licking within bouts. In Experiments 1, 2, and 4, pregnanolone reduced the intrabout lick rate at the highest dose (10 mg/kg). It has been shown that rats lick at a reasonably constant rate of about six to seven licks per second (29) and disruption of this rate is indicative of a deficit in sensorimotor coordination. Therefore, the decrease in intrabout lick rate caused by pregnanolone may have resulted from the sedative effects of this drug (31). This suggests that although pregnanolone did not affect total intake or the structure of bouts of licking for sucrose, it was, nevertheless, having a pharmacological effect.

The effect of pregnanolone on consumption of a sweet wet mash diet was examined to control for the possibility that the failure to demonstrate a hyperphagic effect of this drug was due to the specific orosensory properties and/or postingestive consequences of the sucrose test meal (Experiment 3). Pregnanolone did not significantly affect intake of the mash diet. These data suggest that the lack of effect of pregnanolone on food intake is not limited to carbohydrate liquid diets but also extends to solid diets of mixed macronutrient composition.

The results of Experiments 1–4 are not consistent with a recent report of the effects of pregnanolone on ingestive behavior (6). These authors found that pregnanolone, and two other 3α-hydroxylated pregnane steroids, allopregnanolone and alphaxalone, increased intake of sweetened chow pellets in nondeprived male rats. One difference between the method used by Chen et al. (6) and that employed in Experiments 1–4 was the amount of training the rats received before testing. The rats in the Chen et al. studies received only 1 day of familiarization, whereas in Experiments 1–4 the rats underwent at least 7 days of training. This difference was controlled for in Experiment 5, which tested the effect of pregnanolone in a 10-min food preference test. Rats were given a choice between both novel and familiar items, without undergoing prior training. Under these conditions, pregnanolone increased the total log time spent eating, and this was due entirely to an increase in the time spent eating the novel food. The finding that pregnanolone did not affect the time spent eating the familiar food is consistent with the lack of effect of this drug on intake of the familiar sucrose and mash in Experiments 1–4. It has been shown that repeated experience with the food preference test causes a shift in rats' preference for familiar chow towards new foods, probably due to a reduction in anxiety associated with novelty (28). Therefore, pregnanolone may have acted in a fashion similar to repeated exposure, increasing eating time as result of a reduction in the neophobia aroused by the unfamiliar food. This conclusion is consistent with the previously documented anxiolytic effects of pregnanolone (5,31), and suggests that the increase in food intake observed by Chen et al. (6) may have been due to antineophobia.

The effect of pregnanolone on ingestive behavior observed in the present experiments differs from the well-documented effects of BZR agonists in similar tests. Increases in intake of both palatable sucrose solutions and wet mash in familiarized rats have been reported on many occasions (9,11–13). We have also recently shown that the BZR agonist midazolam increases the total number of licks and mean bout duration for various concentrations of sucrose in a brief contact test (20). The effect of the BZR agonist chlordiazepoxide (CDP) in a food preference test has also been reported previously (8). CDP increased the total time spent eating in a 10-min preference test, but unlike pregnanolone, this was due to an increase in time spent eating the familiar food. This result is consistent with the proposal that BZR agonists directly enhance feeding behavior and is the exact opposite of the effect of pregnanolone observed in Experiment 5. These data suggest that the effects of pregnanolone on ingestive behavior can be dissociated from the effects of BZR agonists.

The neurochemical mechanisms involved in the antineophobic effects of pregnanolone are not clear, but some speculations can be made. Chen et al. (6) found that the effect of pregnanolone on sweetened pellets was blocked by pretreatment with the $GABA_A$ antagonist picrotoxin, but not by the BZR antagonist flumazenil, suggesting that modulation of $GABA_A$ transmission at an allosteric site different from the BZR may have a role to play. Assuming this to be the case, it would appear that positive allosteric modulation of $GABA_A$ function whether by pregnanolone or BZR agonists does not necessarily lead to similar behavioral outcomes. Although enhancement of $GABA_A$ transmission by both pregnanolone and BZR agonists can result in anxiolytic, anticonvulsant, and sedative effects, a specific effect on appetite would seem to be limited to BZR agonists. The reason for this may due to the heterogeneity of the $GABA_A$ receptor complex that comprises subunits with multiple isoforms [see (24)]. Different populations of $GABA_A$ receptors probably exist in vivo, and stimulation of a specific population could result in selective behavioral effects. Therefore, the lack of effect of pregnanolone on appetite may be due to the existence of specific $GABA_A$ receptor subtypes that are responsible for modulating feeding behavior, but are insensitive to pregnanolone. However, this hypothesis remains to be fully tested.

In summary, the neuroactive steroid pregnanolone failed to stimulate an increase in food intake or affect the microstructure of licking in well-trained male rats under a variety of experimental conditions. An increase in the time spent eating novel food items was observed in a food preference test that required no prior training. These results suggest that pregnanolone may not have a direct effect on ingestive behavior, but, under certain conditions, may facilitate feeding secondarily due to an antineophobic action. Further work is currently being undertaken to determine if this effect of pregnanolone is representative of other 3α-hydroxylated pregnane steroids such as allopregnanolone and alphaxalone.

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