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Discriminative Stimulus Properties of Pentobarbital and Progesterone in Male and Female Rats

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HEINSBROEK, R P W , F VAN HAAREN, F ZANTVOORD AND N E VAN DE POLL *Discriminative stimulus properties of pentobarbital and progesterone in male and female rats* PHARMACOL BIOCHEM BEHAV **28**(3) 371–374, 1987.—Intact male and ovariectomized female rats were trained to discriminate 12 mg/kg pentobarbital from physiological saline Generalization tests with different doses of pentobarbital did not reveal significant sex differences in the pentobarbital generalization gradient Different doses of progesterone produced a generalization gradient to pentobarbital in ovariectomized females, but not in intact males The results of the present experiment thus suggest that systemic administration of progesterone produces a "pentobarbital-like" stimulus in ovariectomized female rats, but not in intact males

Pentobarbital Progesterone Drug-discrimination Male and female rats

HORMONE-behavior interactions are reciprocal; changes in hormonal conditions affect behavior and in return, behavioral consequences are known to alter endocrine functioning [6]. Dynamic relations between hormones and behavior provide mechanisms by which hormones can determine or modulate the effects of environmental contingencies upon behavior One such mechanism might be state-dependent learning; a particular change in hormonal variables becomes an essential part of a stimulus complex and future behavior will depend upon the presence or absence of this stimulus complex Stimulus properties of hormonal conditions were actually demonstrated by Stewart *et al* [11] using a drug discrimination (DD) procedure

Stewart *et al* [11] successfully trained ovariectomized females to respond differentially in the presence of progesterone or its vehicle. A very high dose of progesterone was used while subjects were specifically trained on the well known sedative effects of high doses of progesterone [4]. Actually, training under progesterone conditions was started "as soon as signs of sedation were evident" [11]. Central sedatives constitute a category of drugs which are readily discriminable in DD procedures [1] If progesterone shares common properties with these centrally acting sedatives, then animals trained to discriminate a particular sedative drug may be likely to generalize progesterone to this drug Successful generalization of progesterone to a central sedative would establish one particular aspect of the progesterone cue. The present experiment was therefore designed to investigate whether administration of different doses of progesterone would generalize to the central sedative pentobarbital. Discriminative stimulus properties of progesterone have previously been established in ovariectomized female rats [11]. Consequently, studying progesterone generalization to pentobarbital was started by using ovariectomized female rats The sedative or anesthetic action of progesterone is known to be less effective in male rats compared to female rats [5] Progesterone generalization was therefore also studied in a group of male rats If progesterone is generalized to pentobarbital, a difference in the dose-generalization gradient is expected between both groups

METHOD

Subjects

Six male and six female Wistar rats were obtained from the Animal House (TNO, Zeist, The Netherlands). They were 12 weeks old upon arrival in the laboratory Subjects were group-housed and maintained under a reversed light dark cycle (lights on from 3 30 p m. to 3.30 a m.) Experiments started when the animals were 16 weeks old. Females were ovariectomized 20 days prior to experimentation Surgery was conducted under fentanyl anesthesia (Hypnorm· 0 1 cc/rat, 0.02%). All animals were handled daily during 10 days preceding the start of the experiment. Subjects were maintained on a 23 hour food deprivation schedule [3] beginning 7 days prior to the first adaptation session Water was always available in homecages

Apparatus

Experiments took place in four standard, Grason-Stadler (model 1111-L) rodent operant conditioning chambers The floor consisted of 23 stainless steel grids, spaced 1.25 cm apart. Two non-retractable levers were located 10 cm above the floor on both sides of a pellet retrieval unit A force in excess of 0 20 N was needed to depress the lever; reinforce-

ment consisted of a 45 mg food pellet (Noyes). Stimulus lights were located slightly to the side and above the levers A houselight was located in the upper left-hand corner of the intelligence panel. Test chambers were enclosed in a sound attenuated compartment (Grason-Stadler model 1101) with a fan to provide fresh air Programming of the experimental conditions and data acquisition was accomplished using the Grason-Stadler 1200 series of programming equipment, located in the experimental room itself.

Procedure

Animals were adapted to the experimental conditions and were subsequently trained to respond on a variable interval 20 seconds (VI20) schedule of food reinforcement After a total of 20 pretraining sessions, discrimination training was started. The VI20 schedule was started after a subject had emitted 20 initial responses, and lasted until a total of 60 reinforcers had been obtained or when the maximum session duration of 25 minutes had elapsed During every session the following data were collected. responses on the left and the right lever preceding the first reinforcer, responses on the left and right lever following the first reinforcer and the total number of reinforcers obtained Only the responses preceding the first reinforcer were used to calculate the percentage of correct responses (number of responses on the lever appropriate to the subject's condition as a percentage of the total number of responses). To eliminate possible influences of olfactory cues, the appropriate lever was varied for animals successively trained in the same conditioning chamber.

Animals were injected daily with either a pentobarbital solution (12 mg/kg body weight) or with just the vehicle (physiological saline). For half of the animals pentobarbital treatment corresponded with training on the left lever, for the other half with training on the right lever Drug condition and vehicle condition were alternated during the initial 40 discrimination training sessions (7 days a week), thereafter, conditions were varied according to a quasi-random schedule (A-A-B-B-A/B-B-A-A-B) Training on a quasi-random schedule was conducted 5 days a week (Monday through Friday) Generalization tests were conducted on Wednesdays and Fridays and started after an individual test criterion had been achieved: at least 40 sessions on the quasi-random schedule and 10 successive sessions (including 5 drug and 5 vehicle days) with the percentage of correct responses being higher than 80% Subjects were allowed to emit a total of 30 responses during a test session. Reinforcers were not delivered, the 30th response ended the session, after which subjects were immediately replaced in their home-cage. Five doses of pentobarbital (1, 2, 4, 8, 16 mg/kg body weight) were tested, followed by 5 doses of progesterone (0, 10, 20, 40, 80 mg/kg body weight) Doses were presented in a quasi-random order to different subjects Table 1 presents an overview of the different treatment conditions. Baseline discrimination performance was determined by averaging performance under pentobarbital training conditions and saline conditions during sessions intervening between the administration of different generalization doses

Drug and Hormone Administration

Sodium pentobarbital solutions (OPG, Utrecht, The Netherlands) were made by diluting pentobarbital in physiological saline. Pentobarbital and physiological saline were injected intraperitoneally (IP), 15 minutes before starting the experiment, in a volume of 0 4 ml/kg. Progesterone (Organon, Oss, The Netherlands) solutions of 10, 20 and 40 mg/ml (testdosages, 10, 20 and 40 mg/kg) were made by diluting progesterone in oil Heating to 60–70 Centigrade for 1–2 hours was applied to speed up dissolving A progesterone concentration of 80 mg/ml (testdosage, 80 mg/kg) was suspended in oil Progesterone was injected IP, 30 minutes preceding experimentation, the injection volume being 1.0 ml/kg

TABLE 1

SEQUENCE OF TEST DOSAGES (mg/kg) OF PENTOBARBITAL AND PROGESTERONE FOR ANIMALS TRAINED TO DISCRIMINATE PENTOBARBITAL (12 mg) FROM PHYSIOLOGICAL SALINE

Subject*	Pentobarbital	Progesterone
1,4	4 8 16 1 2	40 20 10 0 80
2,5	16 1 2 4 8	80 0 40 20 10
3,6	1 2 4 8 16	0 10 80 20 40

*Representing male and female subjects

RESULTS

Acquisition of pentobarbital discrimination was evaluated by averaging the percentage of correct responses for every block of 10 successive sessions Acquisition data from the first 5 blocks (50 sessions) were analyzed using analysis of variance with SEX and BLOCK (repeated measure) as main factors Only BLOCK was found to be significant, $F(4,40)=36\ 55$, $p<0.001$ Thus, male and female rats acquired the pentobarbital discrimination equally fast. The 5th block of training sessions consisted of the first 10 sessions during which conditions were no longer alternated, but varied according to a quasi-random schedule. The average percentage of correct responses during the 5th block was 88 3%, during the preceding 4th block this percentage was 85.6% Apparently, changing from the alternation schedule to the quasi-random schedule did not result in a decrement in performance

The number of responses per reinforcer was used as an index of activity The mean number of responses per reinforcer was calculated separately for pentobarbital and saline conditions (5 sessions for each condition in every block of 10 sessions) Data of the first 50 acquisition sessions were analysed using a 3-way analysis of variance, with SEX, CONDITION (pentobarbital or saline) and BLOCK as main factors Both CONDITION and BLOCK were repeated measures Males responded more often per reinforcer compared to females, males 15.2, females 9 5, SEX, $F(1,10)=7.39$, $p<0\ 05$, whereas during pentobarbital conditions both males and females were more active compared to saline conditions, pentobarbital. 14.1, saline 10 6, CONDITION, $F(1,10)=18.88$, $p<0.001$. The total number of reinforcers per session was averaged in the same way and also subjected to analysis of variance (main factors SEX, CONDITION and BLOCK). Males obtained an average of 58 reinforcers per session and females an average of 55 reinforcers per session, SEX, $F(1,10)=6\ 69$, $p<0.05$ Differences between pentobarbital and saline were essentially unaltered after 100 training sessions Analysis of variance, conducted on the mean number of responses per reinforcer during the

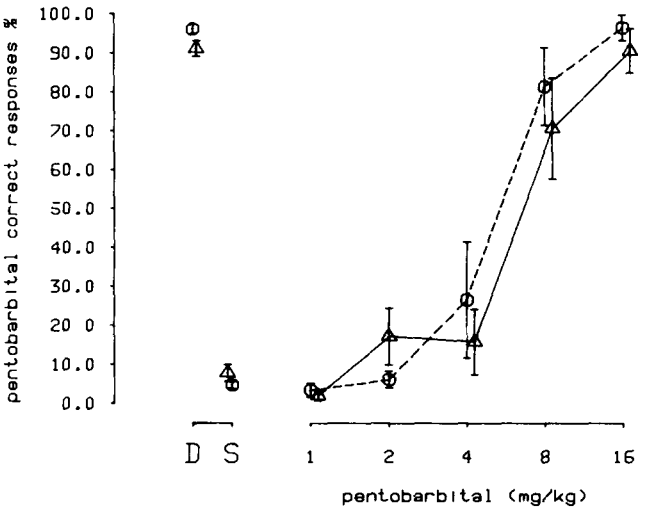

FIG 1 Dose-response curve of pentobarbital for intact males (△) and ovariectomized females (○) trained on discrimination of pentobarbital (12 mg/kg) from physiological saline Averaged percentage of pentobarbital correct responses are presented (+S E) D baseline performance during pentobarbital training sessions, S baseline performance during saline training sessions

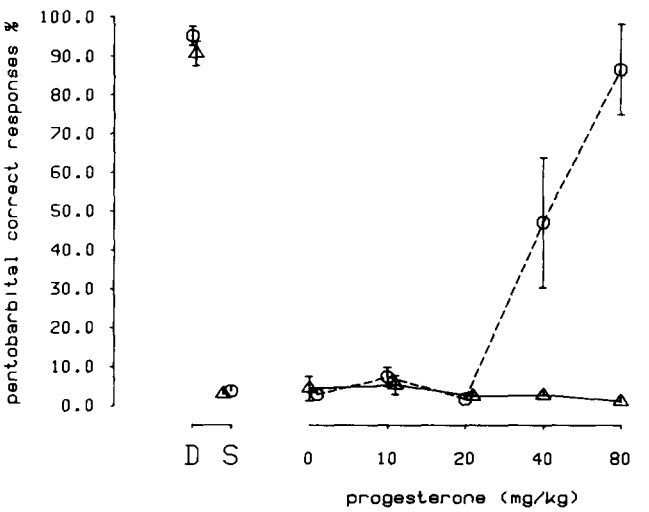

FIG 2 Dose-response curve of progesterone for intact males (△) and ovariectomized females (○) trained on discrimination of pentobarbital (12 mg/kg) from physiological saline Averaged percentage of pentobarbital correct responses are presented (+S E) D baseline performance during pentobarbital traning sessions, S baseline performance during saline training sessions

10th block (main factors SEX and CONDITION), again showed a significant effect of CONDITION, pentobarbital· 16.4, saline. 11 0; F(1,10)=11 25, $p<0$ 01 SEX was no longer significant. Analysis of variance of the mean number of reinforcers per session during the 10th block (main factors: SEX and CONDITION), failed to show any effect On the average males obtained 60 reinforcers per session and females 57 reinforcers per session during the 10th block of discrimination training

Testing animals with a number of different dosages of pentobarbital revealed dose-response curves shown in Fig 1 The baseline performance of males and females (based on 10–20 intervening training sessions) was compared using t-tests (always two-tailed); significant differences were not evident during the pentobarbital condition nor during the vehicle condition ($p>0$ 05). In order to evaluate possible group differences in dose generalization gradients, test scores were subjected to analysis of variance with SEX and DOSE (repeated measure) as main factors A significant effect of the main factor DOSE was observed, F(4,24)=29 83, $p<0$ 001 however, SEX and the SEX by DOSE interaction were not significant

Results of the progesterone tests are shown in Fig 2 t-Tests conducted on the pentobarbital and saline baseline discrimination performance (during 10–20 intervening training sessions) did not reveal significant differences between females and males ($p>0$ 05). The progesterone test scores were subjected to analysis of variance with SEX and DOSE (repeated measure) as main factors SEX was significant, F(1,10)=32 22, $p<0$ 001, DOSE was significant, F(4,40)=17.47, $p<0$ 001, and the interaction between SEX and DOSE was significant, F(4,40)=19 95, $p<0.001$. Progesterone dose-dependently affected test scores in ovariectomized females, but not in intact males (Fig. 2) Test scores were subsequently compared with baseline saline performance using paired t-test analysis. In the group of males, test scores and baseline saline performance did not show significant differences ($p>0.05$) For ovariectomized females however, 40 mg/kg and 80 mg/kg progesterone did result in a significantly different performance compared to the saline training condition, t(5)=−2 67, $p<0.05$ and t(5)=−7 31, $p<0$ 005, respectively

DISCUSSION

In the present experiments, ovariectomized female rats trained to discriminate 12 mg/kg pentobarbital from physiological saline, were observed to generalize test doses of progesterone to pentobarbital. In other words, ovariectomized females responded differentially based on an internal condition evoked by progesterone treatment, confirming earlier observations concerning internal stimulus properties of progesterone [11]. However, the present results point to similar stimulus properties of pentobarbital and progesterone Progesterone can thus exert control over behavior within a DD paradigm based on properties shared with central sedatives and this stimulus control is likely to be evident at doses at least as low as 40 mg/kg.

Contrary to ovariectomized females, intact males trained on pentobarbital discrimination did not generalize pentobarbital to progesterone doses used in this experiment Female rats are well known to be more susceptable to pentobarbital than male rats, recently confirmed by a longer duration of pentobarbital induced sleep [13] as well as a stronger induction of physical dependence on pentobarbital in females [12] Moreover, rate decreasing effects of pentobarbital on schedule-controlled behavior were found to be stronger in ovariectomized female compared to intact male rats (Heinsbroek *et al*, in press) However, despite sex differences in pentobarbital susceptability, significant sex difference were not observed in the acquisition of pentobarbital discrimination, nor in the generalization gradient of pentobarbital Therefore, the absence of progesterone generalization found in intact males cannot be attributed to sex differences in pentobarbital susceptability

Activity increasing effects of pentobarbital were observed

in males and females; under pentobarbital conditions animals emitted more responses per reinforcer as compared to saline conditions The same dose of pentobarbital used to train animals in the present experiment (12 mg/kg), clearly suppressed behavioral output of both ovariectomized female and intact male rats trained on a random ratio (RR) 20 schedule of food reinforcement (Heinsbroek *et al* , in press) The presently observed increase in activity during pentobarbital sessions is likely to be a consequence of both training requirements and chronic pentobarbital treatment. Similar to pentobarbital progesterone has also been found to increase schedule controlled responding Response rates of ovariectomized females trained on RR 20 were increased after progesterone treatment. However, response rates of intact males were not affected by progesterone (Heinsbroek *et al* , in press). These data again show that progesterone has properties in common with pentobarbital and again these properties were specifically manifested in females.

The stronger anesthetic action of progesterone in female rats compared to male rats [5] may be relevant to the observed sex difference in progesterone generalization The sex difference in anesthetic action of progesterone was related to differences in metabolic activity in liver microsomes, resulting in differences in tissue levels of progesterone and progesterone metabolites between female and male rats [5] Anesthetic potency varies among progesterone metabolites [4,5] and therefore, a sex difference in metabolic activity could explain the sex differences in anesthetic action of progesterone and might result in the presently observed sex difference in progesterone generalization. The relevance of progesterone metabolites for generalization to pentobarbital is further suggested by the finding that the metabolite 3α-hydroxy-5α-dihydroprogesterone affected GABA receptors in a similar way as pentobarbital does [8] In addition, progesterone altered the responsiveness of cerebellar Purkinje neurons to GABA [10] and increased GABA binding [7] A GABA receptor complex is thought to be of relevance for the central effects of barbiturates and benzodiazepines [9]. GABA receptors have also been implicated to be involved in the discriminative stimulus properties of both barbiturates and benzodiazepines [2]. Therefore, a cue function of progesterone may be related to interactions of progesterone or progesterone metabolites with the GABA receptor complex

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