Pharmacology Biochemistry & Behavior, Vol. 4, pp. 497–505.

Effect of CNS Depressants and Stimulants on Latency for the Appearance of Copulatory Response in the Female Rat

HUGO CARRER AND BENGT J. MEYERSON

Department of Medical Pharmacology, Biomedicum, Box 573
S-751 23 Uppsala, Sweden

(Received 27 October 1975)

CARRER, H. AND B. J. MEYERSON. *Effect of CNS depressants and stimulants on latency for the appearance of copulatory response in the female rat.* PHARMAC. BIOCHEM. BEHAV. 4(5) 497–505, 1976. – Diethyl ether anesthesia, sodium hexobarbital (20 mg/kg), diphenylhydantoin (50 mg/kg), strychnine (1 mg/kg) and picrotoxin (1–0.25 mg/kg) effectively induced the copulatory response (lordotic behavior) in estradiol benzoate treated ovariectomized rats although no progesterone was given. As none of the tested compounds were effective in replacing progesterone in adrenalectomized animals, adrenal secretion is likely to be implicated in the lordosis activating effect of these compounds. The lordosis response appeared faster after the CNS stimulants than after treatment with the CNS depressants. The influence of diethylether anesthesia, strychnine (0.5 mg/kg) or picrotoxin (0.25 mg/kg) on the latency for the appearance of the lordosis response after IV injection of isopregnenone was studied in estradiol benzoate treated ovariectomized females. A 10 min ether anesthesia delayed the onset of the lordosis response in adrenal intact as well as adrenalectomized animals. Anesthesia given after receptivity had been fully established suppressed the responses for a short period (10–30 min) after the narcosis. The delay of the appearance of the first lordosis response after IV injection of isopregnone exceeded this period. Strychnine but not picrotoxin significantly shortened the latency to the onset of the female copulatory response. It is concluded that the lordotic activating action of progesterone or steroids with progesteronelike ability released from an endogenous source or given IV is influenced by compounds which exert a depressant or stimulant effect on neuronal activity. The total response obtained is not changed but the appearance of the response is prolonged by CNS depressants and shortened by certain CNS stimulants.

Female copulatory behavior Adrenalectomy Progestin treatment Ether anesthesia
Sodium hexobarbital Diphenylhydantoin Strychnine Picrotoxin

COPULATORY behavior in the ovariectomized rat, the lordosis response on mounting by a male, is restored more effectively by estrogen followed by progesterone than by estrogen alone [2, 4, 19, 20, 25, 32]. A direct central nervous effect of progesterone is suggested from radiochemical and implantation studies. Progesterone is taken up and retained in a variety of different regions of the rodent brain [27, 28, 30, 33]. Implants of progesterone into the mesencephalic reticular formation and medial, basal hypothalamus facilitate lordotic behavior, however, the results from different laboratories as to which area is the critical site for the progesterone action are not completely in agreement [24,26]. Very little is known about the central nervous mechanism by which progesterone activates lordotic behavior in the estrogen-primed female rat. Increasing physiological and pharmacological evidence suggest that there exist pathways mediating an inhibitory control of the copulatory behavior in the female rat (evidence reviewed in [3, 12, 18, 21]). It is not clear which role progesterone plays in this respect. Hypothetically, progesterone might remove an inhibition or act synergistically with excitatory functions to override a tonic inhibition. An antagonistic action on lordotic behavior of increased monoaminergic activity has been proposed, based upon the effect of neuropharmacological agents with different actions on central nervous monoaminergic mechanisms [21]. Compounds which increase the monoaminergic activity were found to inhibit the estrogen + progesterone activated lordosis response. There is at present evidence that serotonergic, dopaminergic and cholinergic (muscarinic) pathways are involved in the mediation of this inhibitory effect [9, 13, 14, 16, 18, 22]. Compounds which decrease monoaminergic activity were found to facilitate the lordosis responding in female rats treated with estrogen alone [17]. Also cortical application of potassium chloride would replace the progesterone treatment [6]. These effects could to a certain extent be ascribed to endogenous adrenal progestin release, achieved by the drug treatment [1, 8, 23]. However, other data suggest a direct central nervous action of these compounds for the production of the lordosis response [9, 31, 34]. A direct relationship between the progesterone action on lordosis response and transmitter functions is, however, still uncertain.

The reasoning behind the present study was that in

addition to studies of drug effects on the amount of lordosis responding, activated by estrogen + progesterone, or substitution of progesterone with neuroactive agents, a further insight into the problem of the mechanisms of progesterone action could be obtained by investigating the influence of neuropharmacological compounds on the latency to appearance of the progesterone-activated lordosis response. The specific objectives of the study were (1) to investigate if the time it takes endogenously released progestin to activate the lordosis response is influenced by the neuronal depressive or stimulative quality of agents used to provoke a progestin-secretion; (2) to investigate how psychoactive drugs, which are not able to substitute for progesterone in activating lordosis in adrenalectomized female rats, influence the latency of the lordosis response to appear after progestin treatment. The rationale is based on the following data arrived at in previous investigations:

Progesterone has general anesthetic properties [19,29]. When the capacity of different progestins to induce lordosis behavior and their anesthetic action were compared no correlation was found between these 2 qualities, indicating that the lordotic behavior was induced by a specific action different from the general anesthetic effect. Furthermore, the latency to appearance of the lordosis response after different progestins given intravenously in estrogen-treated ovariectomized rats was shown to be prolonged by increasing the dose of progestins with inherent anesthetic properties. This was not the case with isopregnenone, a 6α-dehydroretroprogesterone, with no general anesthetic effect. The latency after high doses of progesterone was longer than after equivalent doses of isopregnenone indicating that progesterone has an initial inhibitory action besides the well known activating effect on the lordosis response. Thus, it seems likely that the onset of the response after exogenous progesterone treatment was delayed by the inherent anesthetic property of this progestin. It was therefore of interest to extend these studies to the effect on the appearance of lordosis response after treatment with CNS depressants and CNS stimulants. This was done in order to investigate if the mechanism by which progesterone (endogenous or administered) activates the lordotic behavior is sensitive to a depressive effect on neuronal activity. The CNS-depressing compounds used in the present study were: a general anesthetic, diethylether; a barbiturate, hexobarbital sodium; the antiepileptic compound, diphenylhydantoin and the CNS stimulating agents were strychnine and picrotoxine which selectively block post-synaptic (strychnine) or pre-synaptic (picrotoxine) inhibition respectively.

METHOD

Animals

A total of approximately 400 albino Sprague-Dawley rats weighing 300–350 g were kept under reversed day night schedule (12 hr light–12 hr darkness) at 22–24°C and provided with commercial food pellets and tap water ad lib. The animals were ovariectomized on arrival at the laboratory and tested approximately 3 weeks after surgery. Adrenalectomized animals were supplied with 0.9% sodium chloride throughout the experiment.

Procedure

The tests for lordotic response were performed from 3 to 9 hr after the begininng of the dark period and were performed in dimmed light. The female was brought to an observation cage (40 × 40 × 30 cm) containing a vigorous male rat and each test was ended after 6 mounts by 1 or 2 males. A female was considered to have shown a positive lordotic response when during the test she displayed at least 2 lordoses. The results are expressed as a percentage based on the number of animals which showed positive responses in each test.

A single dose of estradiol benzoate, 10 μg/kg SC was given 1 hr before the dark period started. All other treatments were given 48–54 hr later. The animals were rested for at least 2 weeks between 2 treatments. Animals were subjected to more than 1 treatment but were never used more than once in the same treatment category. Animals used as controls were also included in one of the other treatment categories. Controls were always run at the same test occasion as experimentals. In order to screen animals which were responsive as a consequence of estrogen injection alone, the rats were tested before drug administration. Animals which displayed a positive response were excluded from further testing. When all data are pooled 7% of nonadrenalectomized animals and 11% of adrenalectomized animals were excluded on this basis. An exception to this rule was made when progesterone or isopregnenone were given SC at 48 hr. Latency means the elapsed time from the progesterone injection to the first test in which a positive response was observed.

Statistical Analysis

All experiments were run in at least 2 replicative sessions. Statistical significance was tested by the χ^2 test corrected for continuity or Fisher's exact probability test, if not otherwise stated.

Administered Materials

17β-estradiol benzoate and progesterone (N. Y. Organon, through Erco Ltd, Stockholm, Sweden) were dissolved in olive oil. Isopregnenone (6-dehydroretroprogesterone, Ferrosan, Malmö, Sweden) was dissolved in propylene glycol. Hormones were injected in a volume of 0.25–0.35 ml. Diethyl ether (Skånska Bomullskrut, Ltd, Dösjebro, Sweden) was inhaled with access to air. Hexobarbital sodium (Bayer Pharma, Stockholm, Sweden), strychnine (ACO, Stockholm, Sweden) and picrotoxine (Fluka through Labkemi, Stockholm, Sweden) were dissolved in saline. Diphenylhydantoin (Epanutin®, Parke-Davis, Houneslow, England) was dissolved in propylene glycol 40%, ethanol 40%. Intravenous injections were given in one of the lateral tail veins, injection volume was 0.2 ml.

RESULTS

Experiment 1 Estradiol Benzoate + Drug Treatment

The effect of ether anesthesia and sodium hexobarbital. Estradiol benzoate treated spayed females were given 10 min of ether anesthesia. The animal was placed inside a cylindric glass jar containing a piece of cotton soaked with ether covered by a wire mesh, on top of which the animal was placed. Care was taken to let sufficient air into the jar. The righting reflex was lost after about 1 min whereupon the animal was removed from the jar and the anesthesia maintained by letting the animal breathe fumes from an

TABLE 1

LORDOSIS RESPONSE IN OVARIECTOMIZED RATS AFTER TREATMENT WITH ESTRADIOL-BENZOATE (EB) AND ETHER, SODIUM HEXOBARBITAL, DIPHENYLHYDANTOIN OR PROGESTERONE

Treatment at Time 0	Lordosis Response % Min after Treatment 30	90	180	N
A. 1. Ether anesthesia for 10 min	22	48	70¶	23
2. Controls	9	18	14	22
B. 1. Sodium hexobarbital, 20 mg/kg IP		6	78**	22
2. Sodium hexobarbital, 40 mg/kg IP	5	29	62	21
3. Saline, 0.2 ml		17	13	24
C. 1. Diphenylhydantoin, 50 mg/kg IP	45§	65	80†¶	20
2. Blank solution, 0.2 ml	6	33	33†	21
D. Progesterone, 0.4 mg/rat SC*	85	95	95	21

*Progesterone was given 48 hr after the estrogen treatment and tests conducted 4, 6 and 8 hr after the progesterone injection. Estradiol benzoate (EB), 10 μg/lg SC was given 52–54 hr before Time 0.
†Tested at 120 min. ‡40% ethanole and 40% propylenglycol. Significant difference between experimentals and controls §$p<0.05$, ¶$p<0.01$, **$p<0.001$.

ether soaked piece of cotton placed just in front of the nose of the animal. The time from disappearance of the righting relfex till recovery was about 10 min. Care was taken to keep the animals normotherm throughout the anesthesia. The rectal temperature was checked by a thermistor measuring device (Stantel Thermistor, Type F 22). At the first test for lordosis behavior performed 30 min after the beginning of the ether anesthesia the overt behavior was normal. The control group consisted of animals which were placed in a jar without ether for 2 min. Even though no progesterone was given significantly more animals displayed lordosis response 180 mın after the ether anesthesia treatment than after control treatment (Table 1 A). The animals which displayed lordosis at 30 or 90 min also responded at 180 min, i.e. the response lasted for at least 1.5 hr.

Sodium hexobarbital 20 or 40 mg/kg IP was given at Time 0 (Table 1 B). After 40 mg/kg the righting reflex was abolished for about 30 min. At 90 min the animals were not completely recovered. While sitting in the home cage they appeared slightly sedated with decreased spontaneous locomotor activity. However, the locomotor activity was almost normal during the test situation in the male cage. One hundred eighty minutes after the injection the overt behavior was not different from controls. The 20 mg/kg dose produced a slight sedative effect seen at 30 min but not at 90 min after injection. The sodium hexobarbital treatment was effective to produce lordosis response in 62–78% of the animals tested 180 min after injection.

The effect of diphenylhydantoin. The anticonvulsive compound diphenylhydantoin (50 mg/kg IP) was given at Time 0 (Table 1 C). Tests for lordosis behavior were conducted 30, 90 and 180 min later. No effect of diphenylhydantoin was seen on the overt behavior at any of the tests conducted. Diphenylhydantoin treatment induced sexual receptivity, so that at 30 and 120 min the percentage of positive responses was significantly higher than in blank treated animals. All animals responding at 90 min also displayed lordosis behavior at 120 min. Thus, the readiness to respond lasted for at least 30 min. The response of the controls was slightly higher than in other control groups which might be due to the blank solution given, which contained ethanol.

CNS stimulants: strychnine and picrotoxin. Strychnine and picrotoxin were given IP and the lordosis response tested at 20, 40, 100 and 190 min later (Table 2). About half the number of animals treated were given propylenglycol 0.2 ml IV 10 min after the drug treatment (see below). No difference was seen between propylenglycol injected or noninjected animals so the data of both groups were pooled. Already at 20 min after strychnine 1.0 mg/kg and picrotoxine 0.25 mg/kg a significant number of animals showed lordosis response. After picrotoxin 1 mg/kg the response did not appear until the 100 min test. This delay in appearance of the response is probably due to a nonspecific influence of the drug since some animals in this dose category showed convulsions shortly after the injection and were not completely recovered at the 20 min and 40 min tests. The lower dose did not induce convulsions and no effect was seen on the general locomotor performance. Three animals out of 14 responders in the strychnine group responded only at 1 test, but in all others positive responses were evident in at least 2 consecutive tests.

The latency of the response. In Fig. 1 is shown the disbribution of the first lordotic response. The animals in each treatment category have been classified in 2 groups depending on whether they showed the first positive response at the 30–40 min test or later. After sodium hexobarbital 20 mg/kg only one animal responded at the 30 min test. The general activity was slightly decreased but not enough to prevent a response through impaired motor capacity. Isopregnenone given IV induced lordosis response in 67% of the animals at the tests conducted 10 and 30 min after injection. The occurrence of the first lordosis response after ether anesthesia had a longer latency than after isopregnenone ($\chi^2 = 8.23$, $df = 1$, $p<0.01$) or after strychnine ($\chi^2 = 5.6$, $df = 1$, $p<0.002$). The time distribution of responses after diphenylhydantoin, strychnine or picrotoxine treatment were not significantly different from those observed after isopregnenone.

The effect of CNS depressants and stimulants in adrenalectomized animals. Adrenalectomized animals were more sensitive to the sedative and convulsive effects of the compounds used. Adrenalectomized animals took a longer

TABLE 2

LORDOSIS RESPONSE IN OVARIECTOMIZED RATS AFTER TREATMENT WITH ESTRADIOL BENZOATE AND STRYCHNINE OR PICROTOXIN

Treatment at Time 0	Lordosis Response Min after Treatment 20	40	100	190	N
A. Strychnine, 1 mg/kg IP	33†	44†	44†	56†	27
B. Picrotoxin, 1 mg/kg IP	8	17	100‡	100‡	12
0.25 mg/kg IP	24*	24*	43‡	24	21
C. Saline, 0.2 ml IP	3	3	3	6	36

Estradiol benzoate (EB) 10 μg/kg SC was given 53 hr before Time 0.
Significant difference between experimentals and controls *$p<0.05$, †$p<0.01$, ‡$p<0.001$.

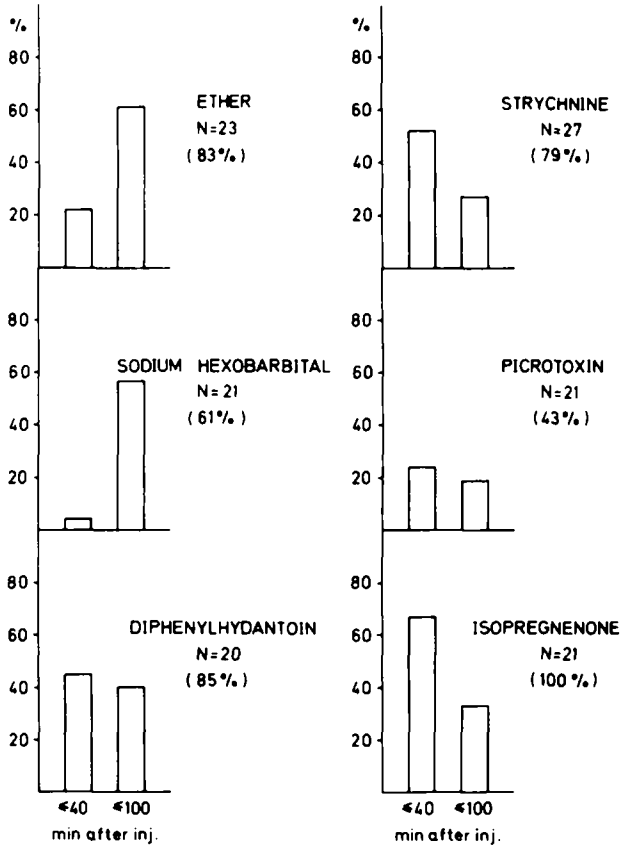


FIG. 1. The latency of the lordosis response. Ether anesthesia was given for 10 min, sodium hexobarbital, 20 mg/kg IP, diphenylhydantoin 50 mg/kg IP, strychnine 1.0 mg/kg IP, picrotoxin 0.25 mg/kg IP, isopregnenone 0.4 mg/rat IV. Estradiol benzoate, 10 μg/kg SC was given 52–54 hr before treatment. In parenthesis cumulated response.

time to recover from sodium hexobarbital, 40 mg/kg. Although no detectable effect of diphenylhydantoin (50 mg/kg) was seen in nonadrenalectomized animals the decrease of locomotor activity was obvious in adrenalectomized animals. The lower dose levels used in adrenalectomized animals were chosen so as not to interfere with the animal's motor capacity. A very slight sedative effect was seen at the 30 min test after the ether treatment and hexobarbital 20 mg/kg.

As shown in Table 3, the slight response induced by ether, hexobarbital or diphenylhydantoin in adrenalectomized estrogen treated animals, was not significantly different from that obtained after saline. At 90 min after diphenylhydantoin 50 mg/kg, 37% of the animals showed an incomplete lordosis response, i.e. they accepted the male, arching the back but did not display the typical pattern of the head posture and the response did not last for a short time after the male had dismounted as is necessary for a full response according to our test criteria. These partial responses were excluded from the data presented in Table 3.

The lordosis response after strychnine, 1.0 mg/kg, was not significantly higher than in saline treated controls.

The capacity to respond was not lost in adrenalectomized animals, since 100% displayed lordosis response when progesterone was given (Table 3 f).

Experiment 2 Estradiol Benzoate + Isopregnenone + Drug Treatment

The effect of diethyl ether anesthesia: ovariectomized females. Progesterone, 0.4 mg/animal, was given SC 48 hr after the estradiol benzoate treatment, 4 hr prior to a 10 min diethyl ether anesthesia. The first test was conducted just after the narcosis. At this time the animals were able to move around in the testing cage, but their motor performance was still sluggish. The lordosis response was decreased when compared with the response obtained before the ether treatment (McNemar test for the significance of changes $p = 0.002$, Fig. 2). At the 30 min test the lordotic response was, however, completely recovered. This was significant, since it shows that 30 min after ether anesthesia the animals had reacquired their motor ability so that they could perform at normal levels. Figure 2 shows the effect of ether anesthesia on the response activated by isopregnenone given IV during the ether anesthesia. The bars of Fig. 2 depict the distribution of latencies. The difference between the distribution of latencies was tested by the χ^2 test. A 2 x 2 contingency table was used with the data from the 30 min test in one column and the pooled data from the 90 + 180 min test in the other one. The appearance of the lordosis response was significantly delayed after the ether treatment ($\chi^2 = 4.23$, $df = 1$; $p<0.05$).

The effect of diethyl ether anesthesia: ovariectomized and adrenalectomized females. The appearance of the lordosis response after intravenous injection of isopreg-

TABLE 3

LORDOSIS RESPONSE IN OVARIECTOMIZED AND ADRENALECTOMIZED RATS AFTER TREATMENT WITH ESTRADIOL BENZOATE IN COMBINATION WITH ETHER ANESTHESIA, SODIUM HEXOBARBITAL, DIPHENYLHYDANTOIN, STRYCHNINE, PICROTOXIN OR PROGESTERONE

Treatment at Time 0	Lordosis Response % 30	90	180	Cumul	N
A. Ether anesthesia for 10 min	0	5	26	26	19
B. Sodium hexobarbital, mg/kg IP					
40	ND	0	0	0	12
20	0	0	0	0	10
C. Diphenylhydantoin, mg/kg IP					
50	7	4	0	7	27
25	16	11	11	21	19
D. Strychnine, mg/kg IP					
1.0	32	26	16	37	19
0.5	27	0	0	27	26
E. Picrotoxin, mg/kg IP					
0.25	0	10	0	10	11
F. Progesterone, mg/rat SC					
0.4	100	100	100	100	24
G. Saline 0.2 ml (Blank solution)	13	10	3	17	30

Estradiol benzoate 10 μg/kg SC was given 53 hr before time zero.
ND = no data. Drug treatment did not differ significantly from saline-controls.

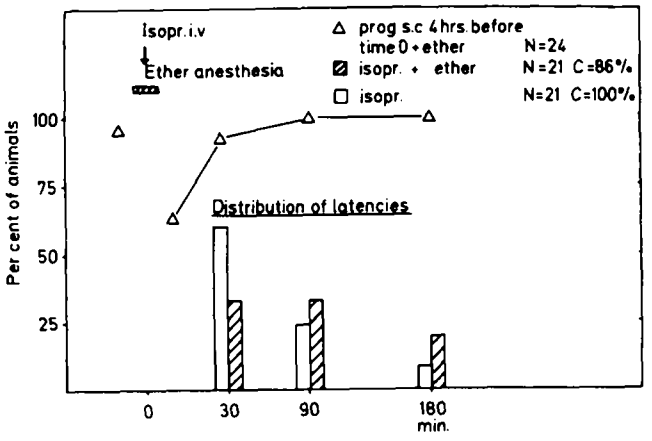


FIG. 2. Lordosis response in ovariectomized rats after ether anesthesia (10 min) and IV injection of isopregnenone. Bars represent distribution of the appearance of first lordosis response after the isopregnenone treatment. Estradiol benzoate 10 μg/kg SC was given 54 hr before Time 0. The SC injection of progesterone was followed by propylene glycol 0.2 ml IV at Time 0. N = number of animals tested. C = cumulative response at 180 min.

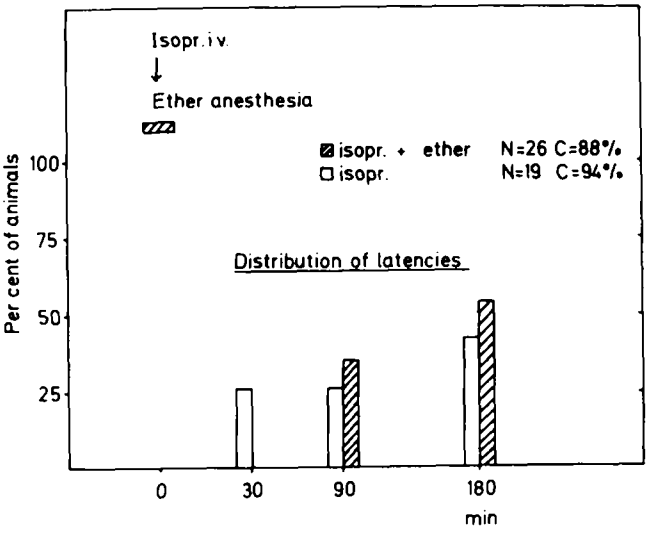


FIG. 3. Lordosis response in ovariectomized and adrenalectomized rats after ether anesthesia (10 min) and IV injection of isopregnenone. Bars represent distribution of the appearance of first lordosis response after the isopregnenone treatment. Estradiol benzoate 10 μg/kg SC was given 54 hr before Time 0. N = number of animals tested. C = cumulative response at 180 min.

nenone was obviously delayed by a 10 min ether anesthesia given at the time of the progestin injection. The animals which had been under ether narcosis did not display lordosis until the 90 min test (Fig. 3). Difference between distribution of latencies: (χ^2 = 9.9, df = 1; $p<0.01$). A certain locomotor sluggishness was seen in the ether-treated animals at the 30 min test, but this effect had disappeared 1 hr later. To study the effect of ether anesthesia on the already developed response isopregnenone (0.5 mg/kg) was given SC. A test was conducted before the ether treatment, and another test 30 or 70 min after the ether anesthesia. The response was significantly reduced 30 min but not 70 min (compared to pre-ether treatment) after the ether treatment (Table 4). A clearcut lordosis response was obtained in 90% of the animals retested 1 hr after the 30 min test. Thus, the established lordosis response was reduced 30 min but not influenced at 70 or 90 min after a

TABLE 4

THE EFFECT OF DIETHYL ETHER ANESTHESIA ON ESTRADIOL BENZOATE + ISOPREGNENONE SC ACTIVATED LORDOSIS RESPONSE IN OVARIECTOMIZED AND ADRENALECTOMIZED RATS

	Lordosis Response %				
	Min before Ether	Min after Ether			N
	30	30	70	90	
Experimentals	85	48*		90	21
	94		81		16
Controls (no ether)	85	95		95	21

The diethyl ether anesthesia lasted 10 min. Estradiol benzoate 10 μg/kg SC was given 48 hr before isopregnenone 0.5 mg/rat SC. The tests before ether treatment were conducted 5 hr after isopregnenone injection. Significance of difference between experimentals and controls $*p<0.05$.

10 min ether narcosis. The response 70 min after ether when the anesthesia was given at different time intervals after the isopregnenone IV injection is shown in Table 5. When ether was given 10 min after isopregnenone the response 70 min after the treatment was slighty reduced relative to controls. The difference, however, did not reach statistical significance. Ether administered 100 min after isopregnenone significantly reduced the response tested 70 min later. The cumulative number of animals responding was in this group significantly different from controls.

The effect of strychnine and picrotoxin treatment. The effect of strychnine and picrotoxin given after isopregnenone was studied in adrenalectomized rats. The latency of the response after the IV injection of isopregnenone alone was the same in this experiment (Fig. 4) and in the ether experiment (Fig. 3). Strychnine (0.5 mg/kg IP) followed 10 min later by IV isopregnenone (0.5 mg) activated lordotic behavior in 75% of the animals already at the 30 min test. The analogous data when saline + isopregnenone was given was 22%. The cumulative response at 180 min was 100% in the strychnine group and 81% in the saline + isopregnenone group. When strychnine was given in an analogous experiment but isopregnenone replaced by propylene glycol blank treatment, the cumulative response was 18% (Fig. 4, legend B). This response could be expected from blank treatment (see Fig. 4, legend A and Table 3).

Picrotoxin (0.25 mg/kg) did not alter the isopregnenone-induced response. In contrast to strychnine the number of females which displayed lordosis response at 30 min after isopregnenone was slightly lower than in controls. A pilot experiment was conducted to study whether picrotoxin (0.25 mg/kg) influenced the already established response tested 40 min after injection. Isopregnenone (0.5 mg/animal) was given 5 hr before the test. All animals (N = 12) that responded at the pre picrotoxin test (83%) also displayed lordosis at the post picrotoxin test. An analogous experiment with picrotoxin (0.5 mg/kg) showed, however, a significant inhibition of the post picrotoxin response.

DISCUSSION

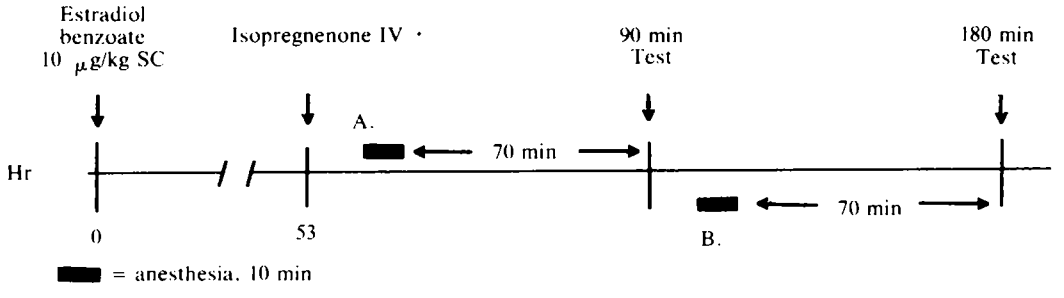
This investigation demonstrates that CNS depressants as well as stimulants elicited the lordosis response in ovariectomized rats more effectively than the estrogen treatment alone. The effective compounds included the volatile anesthetic ether, the barbiturate hexobarbital and the anticonvulsive compound diphenylhydantoin as well as the central stimulant agents strychnine and picrotoxin. Ether,

TABLE 5

THE EFFECT OF DIETHYL ETHER ANESTHESIA ON THE PRODUCTION OF THE LORDOSIS RESPONSE AFTER IV TREATMENT WITH ISOPREGNENONE IN OVARIECTOMIZED ADRENALECTOMIZED RATS

Diethyl Ether Anesthesia	Lordosis Response %			
	min after isopregnenone injection			
min after Isopregnenone, 0.5 mg/rat IV	90	180	cumul. %	N
A. 10 to 20	22	87	87	23
B. 100 to 110	43	43*	54*	28
Controls (no ether)	47	100	100	38

Significance of difference between experimentals and controls $*p<0.01$.



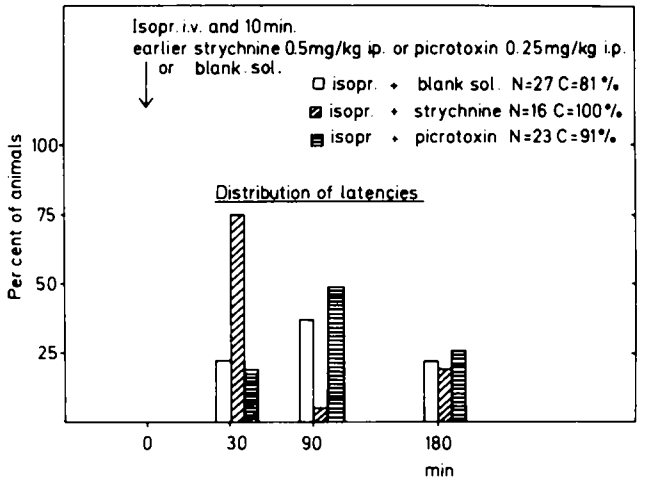


FIG. 4. Lordosis response in ovariectomized and adrenalectomized rats after strychnine or picrotoxin and IV injection of isopregnenone. Bars represent distribution of the first appearance of lordosis response after the isopregnenone treatment. Estradiol benzoate 10 μg/kg SC was given 54 hr before Time 0. N = number of animals tested. C = cumulated response at 180 min. Controls were given the same estrogen treatment but propylene glycol 0.2 ml IV at Time 0 instead of isopregnenone: A (N = 18): 10 min before Time 0, saline 0.2 ml IP. Lordosis response at 30 min 11%, 90 min 5%, 180 min 0%. B (N = 16): 10 min before Time 0, strychnine 0.5 mg/kg IV. Lordosis 30 min 18%, 90 min 5%, 180 min 0%.

diphenylhydantoin and hexobarbital, neither at a time after treatment when some sedation was seen or later when no sedative effect was present, did not produce a significant response in adrenalectomized animals. The doses of strychnine and picrotoxin given to adrenalectomized females were sufficient to influence the general activity, however, the animals were fully capable of adequately participating in the copulatory sequence. The lordosis response obtained in adrenalectomized animals was not significantly different from controls. These results suggest that the lordosis response observed in the nonadrenalectomized animals was induced via adrenal stimulation directly or by means of ACTH release, which increased the output of steroids capable of facilitating sexual behavior. Neuropharmacological agents including ether have been demonstrated to activate adrenal steroid secretion in rats [11,23] (see also introduction). Other possibilities should, however, also be considered: a) that some of the drugs used facilitate the response by an effect directly on the central structures controlling sexual behavior, while the presence of adrenal secretions permit this drug effect to be brought about, b) that the absence of adrenal secretions per se alters central neuronal functions (e.g. secondary to electrolytic or metabolic effects) and in that way changes the effects excerted by the drugs, c) that the high levels of circulating ACTH caused by adrenalectomy impair the drug induced responses. These alternative possibilities are hypothetical and should be experimentally explored.

The data in Fig. 1 indicate that in the nonadrenalectomized animal the CNS stimulants produced their effect with a shorter latency than the CNS depressants. It can be seen that after strychnine or picrotoxin the majority of those animals that became receptive, did so before 40 min had elapsed, while when ether, sodium hexobarbital or diphenylhydantoin were given, most animals became receptive after that time. This difference in latency from injection of the compounds to the appearance of the response could be due to the CNS stimulants either eliciting the lordosis response by a different mechanism of action than the CNS depressants, or have more direct action on the postulated steroid release than ether anesthesia. It seems very unlikely, however, that ether and hexobarbital treatment provoke adrenal progestin secretion with a latency of more than 2 hr. Two min of ether anesthesia increased ACTH secretion in rats within 20 min [11]. A third possibility is that although the CNS depressants produced an endogenous steroid release effective to activate the lordosis response, the response is delayed by the depressant action of these agents. This hypothesis would be in agreement with the previous results on the longer latency after progestins with inherent anesthetic properties relative to the appearance of the behavior after nonanesthetic progestins [20]. It is also in accordance with the fact that an analogous ether treatment as used in Experiment 1 produced in Experiment 2 a delay of the appearance of the lordosis response after isopregnenone (the nonanesthetic progestin) given by IV route. The specificity of the effects obtained in Experiment 2 will now be discussed.

In nonadrenalectomized as well as in adrenalectomized, estradiol benzoate-treated female rats, the latency for the appearance of isopregnenone activated lordosis response was prolonged by a 10 min diethyl ether anesthesia given in connection with the intravenous progestin treatment.

The lordosis response already established by a subcutaneous progesterone or isopregnenone injection prior to ether administration was also influenced by ether; a temporary decrement of the response was obtained in connection with the recovery after the anesthesia. A certain impairment of locomotor activity seen at the tests performed close to the narcosis could be expected to decrease the readiness to respond to mounting by the male.

The question arises whether ether depressed the lordotic response by the same mechanism of action, when given immediately after IV isopregnenone as when it was given 5 hr after SC isopregnenone. Judging from the duration of the effect it would seem that the mechanism was different. In animals anesthetized 5 1/2 hr after receiving SC isopregnenone, the lordotic response, which was fully established before anesthesia, reappeared as soon as the animals regained motor control, i.e., performance was normal at the 70 min test (Table 4). On the other hand, when anesthesia was administered immediately following IV isopregnenone, at a time when isogregnenone was presumably instrumenting its facilitatory influence on receptive behavior, the response took longer to appear (Table 5 A). This was true for both adrenalectomized and nonadrenalectomized animals. This blocking effect of ether anesthesia was most evident when ether was administered after the 90 min test; that is, approximately 100 min after isopregnenone injection (Table 3 B). While at the 180 min test all control animals were receptive, only 43% of the animals which had undergone anesthesia were receptive. No motor impairment can explain this effect, since at this time the animals had fully recovered.

It would then seem reasonable to assume that when ether was administered to animals in which receptivity had already been induced by isopregnenone its deleterious effect on lordotic response was due to the depressed motor performance, but when ether anesthesia intervened at a

time when the effect of isopregnenone was in the process of being established, receptivity was greatly impaired or abolished independently of motor performance. It is concluded that the lordosis activating action of progesterone or steroids with progesteronelike ability released from an endogenous source or given IV, is influenced by compounds which exert a depressant effect on neuronal activity; the total response obtained is not changed but the appearance of the response is delayed. This delay of the response achieved by CNS depression was still obvious at a time after treatment when no overt sedative effect was seen.

After the CNS stimulant compound strychnine, the latency of the isopregnenone-induced response was significantly shorter than after isopregnenone alone. Strychnine alone produced a slight response, however, not different from what could be expected by the estrogen + propylene glycol treatment. Further pharmacological experiments have to be performed to elucidate whether the effect of strychnine is related to a general CNS stimulatory effect or should be specifically ascribed to the block of postsynaptic inhibition achieved by this compound. The fact that picrotoxin, although a CNS stimulant, did not influence the distribution of latencies would suggest that the effect of strychnine is not achieved by a general CNS stimulation. The most obvious difference between the 2 CNS stimulants is that strychnine interferes with postsynaptic inhibition, whereas picrotoxin blocks presynaptic inhibition [5,7]. There is evidence that monoaminergic as well as cholinergic pathways exist which mediate inhibition of the lordosis response (for references see introduction). It is not clear at which level of the CNS these pathways are located. The difference between the effects of strychnine and picrotoxin could possibly be taken as evidence that postsynaptic rather than presynaptic inhibitory functions are involved.

The finding that the latency between the intravenous injection of isopregnenone and the appearance of the lordosis response was longer under adrenalectomy then in nonadrenalectomized animals requires further experiments in order to be explained. Hypothetically, the effect of progesterone could be dependent on electrolytic status and/or metabolic mechanisms which are not adequately functioning after adrenalectomy.

ACKNOWLEDGEMENTS

The authors thank Miss Marita Berg for efficient technical assistance. The work was supported by the Swedish Medical Research Council B74-04X-64-10C. Dr. Carrer was supported by Swedish Institute fellowship.

REFERENCES

1. Ahlenius, S., J. Engel, H. Eriksson, K. Modigh and P. Södersten. Importance of central catecholamines in the mediation of lordosis behaviour in ovariectomized rats treated with estrogen and inhibitors of monoamine synthesis. *J. Neurol. Trans.* **33:** 247–256, 1972.
2. Beach, F. A. Importance of progesterone to induction of sexual receptivity in spayed female rats. *Proc. Soc. exp. Biol. Med.* **51:** 369–371, 1942.
3. Beach, F. A. Cerebral and hormonal control of reflexive mechanisms involved in copulatory behaviour. *Physiol. Rev.* **47:** 289–316, 1967.
4. Boling, J. L. and R. J. Blandau. The estrogen-progesterone induction of mating response in the spayed female cat. *Endocrinology* **25:** 359–364, 1939.
5. Bradley, K., D. M. Easton and J. C. Eccles. An investigation of primary or direct inhibition. *J. Physiol.* **122:** 474–488, 1953.
6. Clemens, L. G., K. Wallen and R. A. Gorski. Mating behavior: Facilitation in the female rat after cortical application of potassium chloride. *Science* **157:** 1208–1209, 1967.
7. Eccles, J. C., R. Schmidt and W. D. Willis. Pharmacological studies on presynaptic inhibition. *J. Physiol.* **168:** 500–539, 1963.
8. Eriksson, H. and P. Södersten. Failure to facilitate lordosis behavior in adrenal-ectomized and gonadectomized estrogen-primed rats with monoamine-synthesis inhibitors. *Hormones Behav.* **4:** 89–98, 1973.
9. Everitt, B. J., K. Fuxe, T. Hökfelt and G. Jonsson. Studies on the role of monoamines in the hormonal regulation of sexual receptivity in the female rat. In: *Sexual Behavior: Pharmacology and Biochemistry*, edited by M. Sandler and G. L. Gessa. New York: Raven Press, 1975.
10. Feder, H. H. and K. B. Ruf. Stimulation of progesterone release and estrous behavior by ACTH in ovariectomized rodents. *Endocrinology* **69:** 171–178, 1969.
11. Guillemin, R. Hypothalamic control of concomitant secretion of ACTH and TSH. In: *The Investigation of Hypothalamic-Pituitary-Adrenal Function*, edited by V. H. T. James and J. Landon. Oxford: Cambridge University Press, 1968.
12. Kow, L. M., C. W. Malsbury and D. W. Pfaff. Effects of progesterone on female reproductive behavior in rats: Possible modes of action and role in behavioral sex differences. In: *Reproductive Behavior*, edited by W. Montagna and A. Sadler. New York: Plenum Publishing Corp., 1975.
13. Lindström, L. H. and B. J. Meyerson. The effect of pilocarpine, oxotremorine and arecoline in combination with methyl-atropine of atropine on hormone activated oestous behaviour in ovariectomized rats. *Psychopharmacologia* **11:** 405–413, 1967.
14. Lindström, L. H. The effect of pilocarpine and oxotremorine on oestous behaviour in female rats after treatment with monoamine depletors or monoamine synthesis inhibitors. *Eur. J. Pharmac.* **15:** 60–65, 1971.
15. Luttge, W. G., R. B. Chronister and N. R. Hall. Accumulation of ^{3}H-progestins in limbic, diencephalic and mesencephalic regions of mouse brain. *Life Sci.* **12:** 419–424, 1973.
16. Meyerson, B. J. The effect of neuropharmacological agents on hormone-activated estus behaviour in ovariectomized rats. *Archs int. Pharmacodyn.* **150:** 4–33, 1964.
17. Meyerson, B. J. Estrus behaviour in spayed rats after estrogen or progesterone treatment in combination with reserpine or tetrabenazine. *Psychopharmacologia* **6:** 210–218, 1964.
18. Meyerson, B. J. Central nervous monoamines and hormone induced estrus behaviour in the spayed rat. *Acta physiol. scand. Suppl.* 241, **63:** 3–32, 1964.
19. Meyerson, B. J. Relationship between the anesthetic and gestagenic action and estrous behavior inducting activity of different progestins. *Endocrinology* **81:** 369–374, 1967.
20. Meyerson, B. J. Latency between intravenous injection of progestins and appearance of estrous behavior in the estrogen treated ovariectomized rat. *Hormones Behav.* **3:** 1–9, 1972.
21. Meyerson, B. J., M. Elliasson, L. Lindström, A. Michanek and A. C. Söderlund. Monoamines and sexual behaviour. In: *Psychopharmacology, Sexual Disorders and Drug Abuse*, edited by T. A. Ban, J. R. Boissier and G. J. Gessa: North Holland Publishing Company, 1973, pp. 463–472.

22. Meyerson, B. J., H. Carrer and M. Eliasson. 5-Hydroxytryptamine and sexual behavior in the female rat. In: *Advances in Biochemical Psychopharmacology II,* edited by E. Costa, M. Gessa and M. Sandler. New York: Raven Press, 1974, 229–242.
23. Paris, C. A., J. A. Resko and R. W. Goy. A possible mechanism for the induction of lordosis by reserpine in spayed rats. *Biol. Reprod.* **4:** 23–30, 1971.
24. Powers, J. B. Facilitation of lordosis in ovariectomized rats by intracerebral progesterone implants. *Brain Res.* **48:** 311–325, 1972.
25. Powers, J. B. and E. S. Valenstein. Individual differences in sexual responsiveness to estrogen and progesterone in ovariectomized rats. *Physiol. Behav.* **8:** 673–676, 1972.
26. Ross, J., C. Claybough, L. G. Clemens and R. A. Gorski. Short latency induction of estrous behavior with intracerebral gonadal hormones in ovariectomized rats. *Endocrinology* **89:** 32–38, 1971.
27. Sar, M. and W. E. Stumpf. Neurons of the hypothalamus concentrate [^{3}H] progesterone or its metabolites. *Science* **183:** 1266–1268, 1973.
28. Seiki, K., M. Higashida, Y. Imanishi, M. Miyamoto, T. Kitagawa and M. Kotani. Radioactivity in the rat hypothalamus and pituitary after injection of labelled progesterone. *J. Endocr.* **41:** 109–110, 1968.
29. Selye, H. Studies concerning the anesthetic action of steroid hormones. *J. Pharmac.* **73:** 127–141, 1941.
30. Wade, G. N., C. F. Harding and H. H. Feder. Neural uptake of (1,2-^{3}H) progesterone in ovariectomized rats, guinea pigs and hamsters: Correlation with species differences in behavioral responsiveness. *Brain Res.* **61:** 357–367, 1973.
31. Ward, I. L., W. R. Crowley and F. P. Zemlan. Monoaminergic mediation of female sexual behavior. *J. comp. physiol. Psychol.* **88:** 53–61, 1975.
32. Whalen, R. E. Estrogen-progesterone induction of mating in female rats. *Hormones Behav.* **5:** 157–162, 1974.
33. Whalen, R. E. and W. G. Luttge. Differential localization of progesterone uptake in brain. Role of sex, estrogen pretreatment and adrenalectomy. *Brain Res.* **33:** 147–155, 1971.
34. Zemlan, F. P., I. L. Ward, W. R. Crowley and D. L. Margules. Activation of lordotic responding in female rats by suppression of serotonergic activity. *Science* **179:** 1010–1011, 1973.