Pharmacology Biochemistry & Behavior, Vol. 4, pp. 521–526.

The Significance of Dopamine, Versus Other Catecholamines, for L-Dopa Induced Facilitation of Sexual Behavior in the Castrated Male Rat

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(Received 17 September 1975)

MALMNÄS, C. O. *The significance of dopamine, versus other catecholamines, for L-DOPA induced facilitation of sexual behavior in the castrated male rat.* PHARMAC. BIOCHEM. BEHAV. 4(5) 521–526, 1976. – The effects of a wide dose range of L-DOPA on male rat sexual behavior were investigated. The animals were castrated as adults and supplied with small amounts of testosterone propionate. It was found that doses of L-DOPA up to 2.5 mg/kg facilitated, while higher doses inhibited, sexual behavior in animals pretreated with pargyline, 20 mg/kg, + MK486, 50 mg/kg. The effects of L-DOPA on sexual behavior were not restricted to the copulatory act, but included elements preceding the copulatory act as well. Most of the facilitatory effects of L-DOPA 2.5 mg/kg were prevented by the dopamine receptor blocker pimozide, 0.10 mg/kg. It is concluded that dopamine is the catecholamine of major importance in mediating the L-DOPA induced facilitation of sexual behavior in the castrated male rat. However, some elements of the copulatory act appear to be modified by noradrenaline and/or adrenaline as well.

Sexual behavior Catecholamines L-DOPA Pimozide

ACCUMULATING evidence suggests that central nervous serotonin inhibits [1, 9, 10, 11, 12, 16] and dopamine facilitates [9, 11, 15] copulatory behavior in the male rat. These concepts are based on experiments with several neuropharmacological agents (monoamine precursors, synthesis inhibitors, receptor blocking and receptor stimulating agents). While the concept of an inhibitory role of serotonin largely has been accepted, the concept of a facilitatory role of dopamine remains controversial. This controversy is due to the fact that the catecholamine precursor L-DOPA has in some studies been found to facilitate [9, 11, 15] and in other ones to inhibit [7,8] male rat copulatory behavior. These studies differ from one another with respect to doses and treatment schedules but share the common feature of having investigated just a very narrow dose range of L-DOPA. Thus, one reason for the divergent results on the effect of L-DOPA on male rat copulatory behavior might be that qualitatively different effects are evoked at different dose levels.

In the present investigation, the effects of a wide dose range of L-DOPA on both precopulatory and copulatory elements of the male rat sexual behavior were investigated. Scoring of motor activity was made parallel to sexual behavior. L-DOPA was given in combination with the monoamine oxidase inhibitor (MAOI) pargyline and the extracerebral decarboxylase inhibitor (DCI) MK486. The effect of pimozide pretreatment on the facilitatory action of L-DOPA was studied as well. Pimozide is a selective blocker of dopamine receptors [2] and the aim was to investigate the significance of dopamine, versus other catecholamines, for L-DOPA induced facilitation of sexual behavior in the male rat.

METHOD

The method has been described in further detail else where [9].

Animals

Male Wistar rats (specific pathogen-free) were purchased from Möllegaard, Ejby, Denmark. The animals had a proven copulatory capacity (at least 1 observed ejaculation) before castration which was performed at 100 days of age. The body weight at the time of experiments had a range between 350–450 g. The animals were housed 3 per cage (Macrolon^{R} 34 × 40 × 15 cm) in a room with forced ventilation at 21 ± 1°C under a reversed day–night light cycle (lights off between 11 a.m.–10 p.m.). Commercial rat pellets (Anticimex 210, Sollentuna, Sweden) and tap water were given ad lib. Stimulus females were spayed Sprague-Dawley female rats treated with estradiol benzoate 25 μg followed 48 hr later by progesterone 1 mg, 4–7 hr before testing with the males.

Procedure

After castration, copulatory tests took place regularly once weekly throughout the entire experimental period. The tests were carried out under dimmed light conditions between 2 p.m.–5 p.m. The male was allowed 5 min of adaptation in the observation cage (40 × 60 × 40 cm with a Plexiglas^R front) before a sexual receptive female rat was introduced. After introduction of the female, the time allowed for the first mount to occur was restricted to 3 min. If the male mounted the female within 3 min the test continued for another 3 min, counted from the first mount, or until ejaculation occurred, whichever came first. When in 2 consecutive weekly postcastration tests less than 50 per cent of a batch of animals mounted the female, testosterone propionate (TP) was given to all of the batch. The TP dose used (0.10 mg/kg/week, 3–4 days before testing) induced a stable, submaximal response with respect to the percentage of males that displayed mounting. This treatment and testing schedule was maintained for two months before the neuropharmacological experiments were performed. Control and experimental animals were tested in parallel. Drugs were only given once to the same animal.

Behavior

The following measures were taken: *Mount percentage*: the percentage of males that displayed at least one mount with or without penile intromission. *Intromission percentage*: the percentage of males that displayed at least one mount with penile intromission. *Ejaculation percentage*: the percentage of males that achieved ejaculation. *Mount latency*: the time from the introduction of the female to the occurrence of the first mount. *Intromission latency*: the time from the introduction of the female to the occurrence of the first mount with intromission. *Number of mounts/min:* total number of mounts (with or without intromission) during the test divided by 3 (ejaculating animals excluded). *Intromission ratio:* $\frac{\text{no. of intromissions}}{\text{total no. of mounts}} \times 100$

In order to evaluate the specificity of drug effects on sexual behavior it is essential to record not only parameters of the copulatory behavior but the occurrence of other behavior patterns as well. For this purpose the occurrence of the following 4 behavior patterns was scored: *Female-oriented activity* (chasing, pursuit and sniffing of the female). *Environment-oriented activity* (exploration of the cage, rearing and other kinds of locomotor activity). *Self-grooming* (genital and nongenital). *Immobility* (standing still or lying down). Every male was given the score of 1 for each of the testing-minutes in which any of these 4 behavior patterns was displayed for at least 0.05 min in a sequence. The number of scores obtained by an animal for a certain class of behavior is expressed as per cent of maximal, i.e. $\frac{\text{no. of scores}}{\text{no. of testing-minutes}} \times 100.$

Drugs

The drugs used were pargyline HCl (^{x}Abbott lab., Illinois), MK486, (^{x}MSD Int., New Jersey), L-DOPA methylester HCl (^{x}Hässle, Sweden), pimozide (^{x}Janssen, Belgium) and the hormones used were testosterone propionate, estradiol benzoate, and progesterone (all from ^{x}Organon, the Netherlands). Hormones were dissolved in olive oil and the drugs, except pimozide, were dissolved in saline. Pimozide was dissolved in glacial acetic acid, diluted with saline and the pH adjusted to 5 by addition of 2 N NaOH. Hormones were given subcutaneously and drugs intraperitoneally, all in a volume of 1 ml/kg. The doses mentioned refer to the forms of the compounds stated above.

RESULTS

The dose-response relationship for L-DOPA, given after pargyline + MK486, is given in Figs. 1a and 1b. Chasing and pursuit of the female, as well as the display of mounting, intromission and ejaculation, were increased by the lower doses of L-DOPA, reaching statistical significance at 2.5 mg/kg. A further increase in the dose of L-DOPA shifted the response into inhibition. After L-DOPA, ongoing activity was sometimes interrupted by periods of immobility during which the subjects were standing completely still for a duration of 0.05–0.5 min. The inhibition of motor activity reached a maximum at 5.0 mg/kg and pronounced inhibition of female oriented activity at this dose-level was mainly due to these motor disturbances. The impairment of motor activity seen after L-DOPA 2.5 mg/kg was about the same as the one after 10 mg/kg and in no case prevented a normal interaction with the female. However, while female oriented activity was increased by 2.5 mg/kg of L-DOPA it was inhibited by 10 mg/kg.

From the dose-response experiment it is apparent that L-DOPA evoked qualitatively different effects on the sexual behavior at different dose levels. The most clearcut facilitatory effects were obtained by L-DOPA 2.5 mg/kg. Therefore, this dose was chosen for investigation of the effect of pimozide pretreatment. The dose of pimozide chosen (0.10 mg/kg) was previously shown not to affect sexual behavior but capable of blocking the facilitatory effects on sexual behavior of the dopamine receptor stimulating agent apomorphine [9]. As shown in Fig. 2, pimozide pretreatment, as such, slightly increased the activity oriented towards the environment (exploration of the cage, rearing, and other kinds of locomotor activity) but did not otherwise affect any of the behavior patterns recorded. However, the increase in female oriented activity induced by L-DOPA was completely antagonized by pimozide. Activity oriented towards the environment was decreased by L-DOPA. Also this effect was completely antagonized by pimozide as was the L-DOPA induced increase in grooming. The impairment of motor functions elicited by L-DOPA was partly antagonized by pimozide. The effect of pimozide treatment on motor activity was further tested in an Animex^R activity meter [14]. The treatments and environmental conditions were identical to the ones used for testing sexual behavior: the animals were simply put into the activity meter instead of into the observation cage. Animals treated with pimozide before L-DOPA had higher motor activity scores than the ones not given pimozide (mean number of counts/10 min ± SEM = 560 ± 22 vs. 492 ± 33, N = 7, $p<0.02$ Mann-Whitney U test).

L-DOPA 2.5 mg/kg facilitated all components of copulatory behavior recorded, except the number of mounts/min which remained unaffected (Table 1). Thus, L-DOPA increased the percentage measures (mount, intromission and ejaculation) and intromission ratio, and decreased the latency measures. Pretreatment with pimozide prevented all facilitatory effects of L-DOPA, except the one on mount latency. Furthermore, when given after

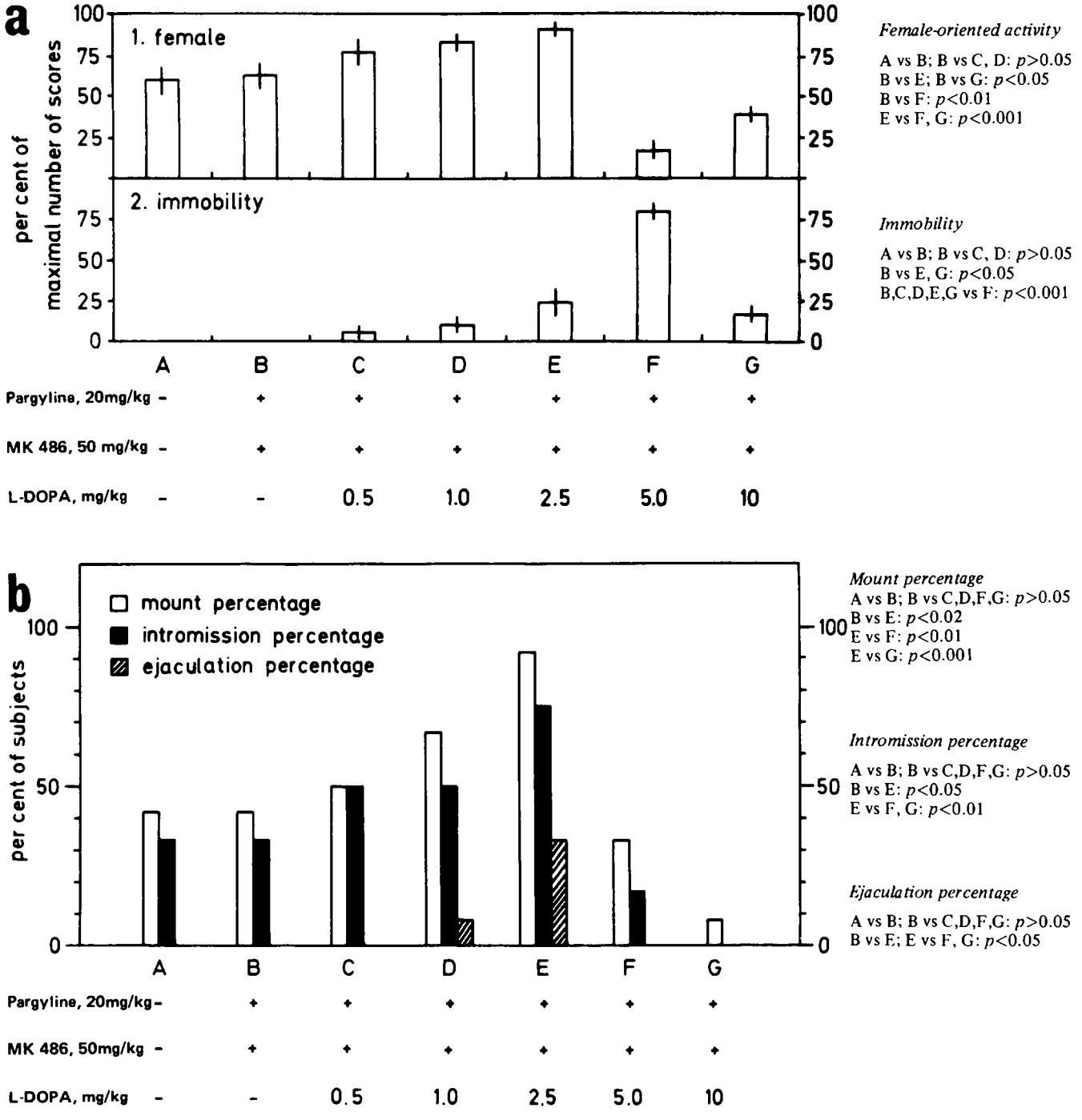
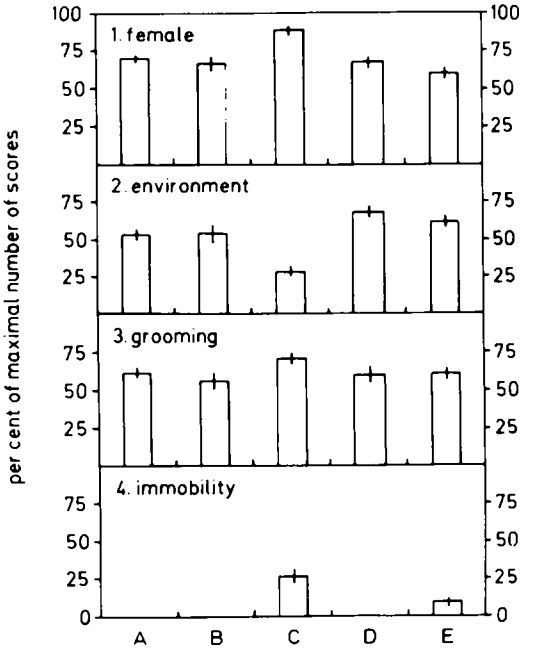


FIG. 1. Dose-response relationship for the effect of L-DOPA on the display of: Fig. 1a: female-oriented activity (1) and immobility (2). Fig. 1b: copulatory behavior. The animals were castrated as adults and supplied with testosterone propionate 0.10 mg/kg/week. Each treatment group was made up of 12 animals, selected on the basis of pre-experimental scores in order to make the groups uniform. Drug and saline injections were given at 1.5 hr (pargyline), 1.0 hr (MK486) and 0.5 hr (L-DOPA) prior to testing. Wherever a minus in the treatment schedule = saline. The significance of differences between groups was tested by means of the Mann-Whitney U test (Fig. 1a) and the Fisher exact probability test (Fig. 1b). The bars in Fig. 1a represent mean ± SEM



Female-oriented activity

A vs B; A vs D; B vs D: $p>0.05$
B vs C; C vs E: $p<0.001$

Activity oriented towards the environment

A vs B; D vs E: $p>0.05$
B vs C: $p<0.001$
B vs D: $p<0.05$
C vs E: $p<0.01$

Grooming

B vs C: $p<0.05$

Immobility

B vs C; D vs E; C vs E: $p<0.001$

FIG. 2. The effect of L-DOPA, with or without pimozide pretreatment, on the display of (1) female-oriented activity, (2) environment-oriented activity, (3) self-grooming, and (4) immobility. The animals were castrated as adults and supplied with testosterone propionate 0.10 mg/kg/week. Drugs and saline were given at 3.0 hr (pimozide), 1.5 hrs (pargyline), 1.0 hr (MK486) and 0.5 hr (L-DOPA) prior to the experimental (= drug) test. Wherever a minus in the treatment schedule = saline. The number of animals in the treatment groups were as follows: A = 94, B = 44, C = 48, D = 42, E = 45. Every animal was given the score of 1 for each of the testing-minutes in which a certain behavior was displayed for at least 0.05 min in a sequence. The bars represent mean + SEM. Probabilities associated with the scores of the different treatment groups were evaluated by means of the Mann-Whitney U test.

pimozide, the number of mounts/min was decreased by L-DOPA.

DISCUSSION

This study demonstrates that while small doses of L-DOPA (≤2.5 mg/kg), given after pargyline + MK486, facilitate sexual behavior in TP treated castrated male rats, higher doses inhibit sexual behavior. Moreover, it is shown that most of the facilitatory effects on sexual behavior of an optimally effective dose of L-DOPA are prevented by pretreatment with the selective dopamine receptor blocker pimozide, given in a dose which by itself does not affect the behavior.

The display of sexual behavior in the male rat consists of highly complex behavior patterns that require a proper motor function. Sexual behavior was inhibited by L-DOPA 5.0 and 10 mg/kg. At the lower of these doses the inhibiton of sexual behavior most likely was due to pronounced motor disturbances. In contrast, the inhibition of motor activity elicited by 10 mg/kg was probably not sufficient to have any major suppressive influence on the sexual behavior since it was of the same order as after 2.5 mg/kg, the dose at which sexually behavior was optimally facilitated. Since high doses of L-DOPA can displace serotonin from central nervous storage sites [4] and extragranular serotonin will not be degradated in the presence of an MAOI, the inhibitory effect of L-DOPA 10 mg/kg on sexual behavior might possibly be due to increased serotonin receptor activity [9].

The increase in female oriented activity, the percentage measures (mount, intromission and ejaculation) and intromission ratio (indicating an improved penile function) elicited by L-DOPA 2.5 mg/kg is prevented by the dopamine receptor blocker pimozide. Thus, it appears that dopamine is the catecholamine of major importance in mediating L-DOPA induced facilitation of these components of sexual behavior in the castrated male rat. In contrast, pimozide does not block the L-DOPA induced decrease in mount latency. Thus, it is possible to dissociate the effect of L-DOPA on mount latency from the one on mount percentage.

Clonidine, an agent which stimulates central nervous noradrenalin (and adrenalin?) receptors [3,5], was previously used as a tool in the investigation of the central nervous regulation of male rat sexual behavior [9]. This substance was found to have 2 main effects on the sexual behavior: a shortening of the mount latency and a decrease of the number of mounts/min. On the other hand, clonidine had no effect on female oriented activity, the percentage measures (mount, intromission and ejaculation) and intromission ratio. It is of great interest that the response elicited by L-DOPA after pimozide (decreased mount latency and no. of mounts/min) is the same as the one elicited by clonidine. The possibility that this effect is due to stimulation of central nervous noradrenalin and/or adrenalin receptors is suggested and should be further elucidated.

Studies on the effects of L-DOPA, after DCI but without MAOI, on various aspects of copulatory behavior in intact male rats have previously been reported [6, 7, 8, 15]. Interpretations of these data are complicated by the fact that it is not clear whether the behavioral effects observed are secondary to an effect on the hypothalamic-pituitary-testicular axis or due to a direct central nervous action.

TABLE 1

EFFECTS OF PIMOZIDE ON L-DOPA INDUCED FACILITATION OF COPULATORY BEHAVIOR IN THE CASTRATED MALE RAT

	Treatment, mg/kg														
				Pargyline, 20 + MK486, 50						Pimozide, 0.10 + Pargyline, 20 + MK486, 50					
	Saline			+ Saline			+ L-DOPA, 2.5			+ Saline			+ L-DOPA, 2.5		
	Pretest	Exp	N	Pretest	Exp	N	Pretest	Exp	N	Pretest	Exp	N	Pretest	Exp	N
Percentage measures															
Mount percentage	56	59	94	59	57	44	54	92‡	48	57	48	42	49	53	45
Intromission percentage	46	48	94	36	34	44	42	65†	48	40	33	42	33	31	45
Ejaculation percentage	0	0	94	0	0	44	0	21†	48	0	2	42	2	2	45
Latency measures*															
Mount latency	0.15	0.15	45	0.15	0.15	22	0.18	0.10†	26	0.15	0.15	17	0.20	0.10†	18
Intromission latency	0.35	0.35	37	0.35	0.43	12	0.30	0.10†	19	0.70	0.50	10	0.20	0.33	10
No. of mounts/min*	2.7	2.7	45	3.2	2.7	22	3.0	3.0	18	2.8	2.5	16	2.7	2.0†	17
Intromission ratio*	53	49	37	43	45	12	56	75†	19	41	57	10	67	57	10

Pimozide was given 3 hr, pargyline 1.5 hr, MK486 1.0 hr and L-DOPA 0.5 hr prior to copulatory tests. Pretest = the test one week prior to the experimental (= drug) test. Pretests represent the baseline-level for the TP induced response. Significance of differences between the behavior in pretest and experimental test was calculated by means of the sign test (percentage measures) and the Wilcoxon matched-pairs signed-ranks test (all except percentage measures). Latencies are expressed in min.

*Md = median. The medians are given only for those animals that displayed the behavior in question both in the pretest and experimental test.

No sign = $p>0.05$; † = $p<0.01$; ‡ = $p<0.001$.

Slight inhibitory effects on copulatory behavior were found by Hyyppä *et al.* [7] and Gray *et al.* [8]. Their findings are in accordance with the ones of the present study that L-DOPA inhibits sexual behavior at certain dose levels. Tagliamonte *et al.* [15] showed that repeated injections of L-DOPA were more effective than a single injection to stimulate male copulatory behavior. Whether this effect was due to the difference in dose or to a more effective serotonin depletion was not made clear. These investigators also demonstrated that the L-DOPA effect was counteracted by the neuroleptic haloperidol. The specificity of this effect could be questioned, however, since the dose of haloperidol used by them (1 mg/kg) most likely made their subjects incapable of copulation. (In our rats, this dose of haloperidol induces a cataleptic state which keeps the males completely immobile for a couple of hr). Another experimental approach was applied by Da Prada *et al.* [6] who found that repeated L-DOPA injections enhanced male-to-male mounting behavior in groups of male rats and they demonstrated the significance of serotonin depletion for this effect. However, like most investigators of male-to-male mounting behavior, Da Prada *et al.* did not show whether the treatment facilitated mounting as such or made the animals more attractive or receptive as mounting objects.

From the present investigation the following conclusions could be drawn. The qualitative effect of L-DOPA on sexual behavior varies. Both facilitatory and inhibitory effects are elicited. The direction of the behavioral change is dependent on the dose level. When given after combined MAOI + DCI treatment, facilitatory effects of L-DOPA on sexual behavior are restricted to quite low doses ($\leq$2.5 mg/kg) while higher doses of L-DOPA inhibit sexual behavior. Most of the facilitatory effects of L-DOPA on sexual behavior (increased chasing and pursuit of the female; increased mount, intromission and ejaculation percentages; decreased intromission latency; increased intromission ratio) are blocked by a small dose of the dopamine receptor blocking agent pimozide. Thus, it appears that dopamine is the transmitter of major importance in mediating the facilitatory effects of L-DOPA on sexual behavior in MAOI + DCI treated castrated male rats. However, evidence is also presented suggesting that dopamine is not the sole catecholamine that affects sexual behavior in the castrated male rat: some components of the copulatory act (notably mount latency and no. of mounts/min) are modified by noradrenalin and/or adrenalin as well.

ACKNOWLEDGEMENTS

For generous supplies of drugs and hormones I thank the companies indicated by an asterisk in the Methods section. The research was supported by grants from the Swedish Medical Research Council (project B73-04X-64-09B).

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