Pharmacology Biochemistry & Behavior, Vol. 14, pp. 17–21. Printed in the U.S.A.

Gonadectomy and Sex Differences in the Behavioral Responses to Amphetamine and Apomorphine of Rats[1]

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Received 21 July 1980

SAVAGEAU, M. M. AND W. W. BEATTY. *Gonadectomy and sex differences in the behavioral responses to amphetamine and apomorphine of rats*. PHARMAC. BIOCHEM. BEHAV. 14(1) 17–21, 1981.—Following treatment with 5 mg/kg d-amphetamine sulfate or 2 mg/kg apomorphine hydrochloride female rats displayed more intense and longer lasting stereotyped behavior than males. Gonadectomy did not affect the display of stereotyped behavior induced by either drug in either sex. A lower dose of amphetamine (1 mg/kg) caused greater stimulation of locomotor activity in females than in males. Castration of males had no effect, but ovariectomy blocked the stimulating effect of amphetamine on activity. By contrast, low doses of apomorphine depressed activity in a dose-dependent manner that was somewhat greater in ovariectomized females than in the other groups. These data add to the growing body of literature demonstrating that gonadal hormones modulate the activity of brain dopamine systems.

Sex differences Gonadectomy Apomorphine Amphetamine Locomotor activity Stereotyped behavior

SEX differences in response to amphetamine treatment have been documented on several behavioral and physiological measures in the rat. For example, female rats exhibit more intense and longer lasting stereotyped behavior than males when given 5 mg/kg doses of amphetamine [3] and lower doses of amphetamine cause greater stimulation of locomotor activity in females [22]. In the present experiment we examined the effects of gonadectomy on these sex differences in response to amphetamine and apomorphine treatment.

METHOD

Animals

Adult rats of both sexes (16 males, 18 females) were purchased from the Holtzman Co., Madison, WI. At about 100 days of age half of the animals of each sex were gonadectomized (Gx) while the others received sham operations or were merely anesthetized. Surgery was performed under Chloropent anesthesia (Fort Dodge Labs, Fort Dodge, IA, 3 ml/kg males, 2 ml/kg females) using clean surgical technique. Except prior to surgery and during behavioral tests the animals had free access to food and water. They were caged singly in an air conditioned animal room maintained at 22±2°C that was illuminated from 0800–2000 hr. Behavioral tests occurred during the light portion of the L:D cycle.

Procedure

Three weeks after surgery the animals were tested for stereotyped behavior after IP injections of 5 mg/kg d-amphetamine sulfate. The rats were placed into standard laboratory cages without food or water and rated for stereotyped behavior every 20 min, commencing 20 min after injection. The 7 point scale devised by Kelly and Iversen [18] was used. On this scale: 0=asleep or stationary, 1=active, 2=active with bursts of stereotyped rearing or sniffing, 3=stereotyped sniffing or rearing over a wide area, 4=stereotyped sniffing or head bobbing in one place, 5=stereotyped behavior in one location with bursts of gnawing or licking, 6=continuous gnawing or licking in one place (including self destructive behaviors such as gnawing the paw or tail).

Ovariectomy reduces dopamine sensitive adenylate cylase but the effect takes 4–6 weeks to develop [19]. Since this is one mechanism by which the behavioral effects of amphetamine might be expressed, the test for stereotyped behavior was repeated 4 weeks later using the same procedure.

Beginning one month later the effects of gonadectomy on amphetamine-stimulation of locomotor activity were studied using the same rats. Shuttling activity was measured in Lehigh Valley Electronics shuttle boxes (Model 143-02) dur-

[1]We thank the SmithKline Corp. and Eli Lilly and Co. for donating the amphetamine and apomorphine used in this study. Please address reprint requests to William W. Beatty.

ing 2-hr long tests that began 10 min after the rats received an IP injection of d-amphetamine sulfate (1 mg/kg) or an equivalent volume of saline. The order of treatments was counterbalanced and at least 7 days intervened between tests.

About 1 month later the effects of apomorphine on shuttling activity were studied. The rats received an IP injection of 0, 0.1, or 1.0 mg/kg apomorphine HCl 5 minutes before a 30 min long test. The order of drug conditions was counterbalanced with at least 3 days between tests. Prior to the start of this phase of the study 1 female from each group developed middle ear disease and both were dropped from the study.

One week later the effect of a 2 mg/kg injection of apomorphine on stereotyped behavior was determined. The procedure was identical to that described above except that 5 minutes elapsed between injection and the first rating and ratings were taken every 10 min for a period of 65 min after injection.

RESULTS

Stereotyped Behavior

The influence of sex and gonadal condition on stereotyped behavior elicited by 5 mg/kg d-amphetamine or 2 mg/kg apomorphine is depicted in Figs. 1 and 2. Since the data from the two tests with amphetamine, conducted 3 and 7 weeks after surgery, were virtually identical, they were pooled. On both tests amphetamine treatment produced more intense and longer lasting stereotyped behavior in female rats (Main effect of Sex: $F(1,30)=34.76$ and 40.91, both $p<0.001$ for Tests 1 and 2 respectively). Although stereotypy scores were somewhat higher in the castrated male group than in the gonadally intact male group, this trend was not statistically significant on either test (Main effect of Gonadectomy and Sex × Gonadectomy interaction: all $Fs<3.05$).

A similar pattern was observed in the tests with apomorphine. Again female rats exhibited more stereotyped behavior than males, $F(1,28)=6.03$, $p<0.025$, but gonadectomy did not affect the magnitude of the drug effect in either sex (Gonadectomy, Sex × Gonadectomy: both $F<1$).

Since the stereotypy scale probably does not meet the requirements of an interval scale, we also analyzed the data with Mann Whitney U tests. Sex differences in stereotypy after amphetamine treatment were observed in intact ($U=0$, $p<0.001$) and gonadectomized animals ($U\leq 14$, $p<0.05$) on both tests), but neither the effect of castration in males ($U=14.5$, $p>0.05$) nor the influence of ovariectomy ($U\geq 36$) was reliable. Nonparametric analyses of stereotyped behavior after apomorphine treatment revealed a sex difference in intact animals ($U=7$, $p<0.01$), but not in gonadectomized subjects ($U=19$). Gonadectomy did not affect the response to apomorphine in either sex ($U\geq 29.5$).

Shuttling Activity

The effects of d-amphetamine and apomorphine on locomotor activity measured in the shuttle box are shown in Table 1. Treatment with 1 mg/kg d-amphetamine stimulated activity in both groups of males as well as in intact females. However, amphetamine did not significantly increase activity in the ovariectomized female group; 4 of the 9 rats in this group were more active after saline injections than after amphetamine treatment. Statistical analysis confirmed the apparent dependence of the drug effect on the sex and gonadal state of the subject. For this analyis difference scores were computed for each animal by subtracting activity under saline from activity after amphetamine treatment. This approach was employed because females were more active than males in the saline condition. Analysis of variance of the difference scores revealed a main effect of Gonadec-

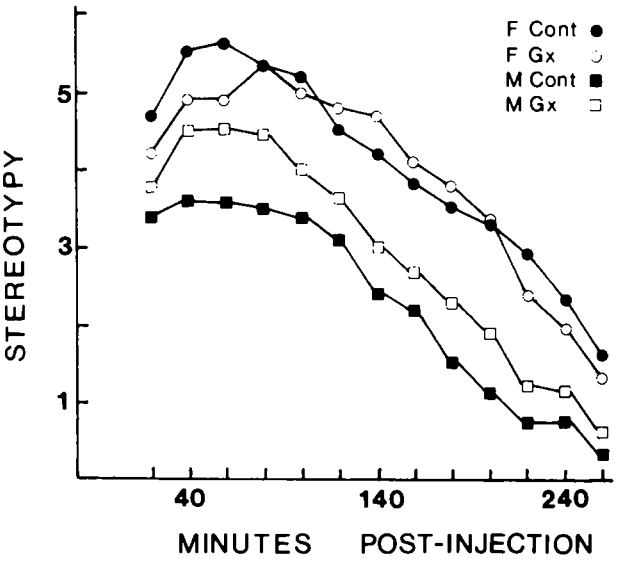

FIG. 1. Mean stereotypy ratings for male and female groups of gonadectomized (Gx) or intact (Cont) animals following treatment with 5 mg/kg d-amphetamine sulfate. Observations began 20 min after injection.

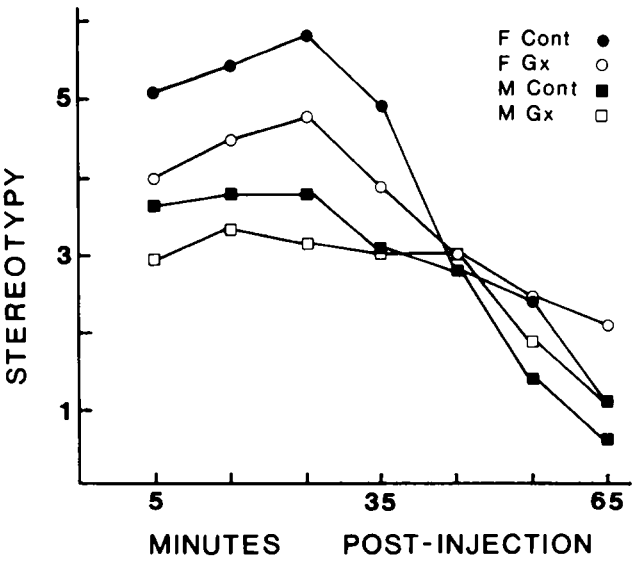

FIG. 2. Mean stereotypy ratings for male and female groups of gonadectomized (Gx) or intact (Cont) animals following treatment with 2 mg/kg apomorphine HCl. Observations began 5 min after injection.

TABLE 1

MEAN NO. SHUTTLING RESPONSES (±SEM)

	2 hr Test		30 min Test		
	Saline	Amphetamine†	Apomorphine (mg/kg)‡		
Group*			0	0.1	1.0
Intact Males	80.9 ± 8.1	202.6 ± 38.4	38.5 ± 3.5	25.3 ± 5.7	14.9 ± 4.5
Gx Males	77.6 ± 7.0	256.0 ± 38.1	30.3 ± 3.5	13.0 ± 2.6	7.5 ± 2.1
Intact Females	104.7 ± 15.9	400.2 ± 49.9	48.0 ± 4.8	32.1 ± 4.0	33.4 ± 7.9
Gx Females	104.0 ± 10.5	154.4 ± 27.5	44.9 ± 4.5	19.4 ± 2.5	11.1 ± 4.4

*Gx=gonadectomized.

†Amphetamine increased activity in every group except the Gx females.

‡Apomorphine reduced activity at both doses in every group except for the intact females at the 1.0 mg/kg dose.

tomy, $F(1,30)=7.86$, $p<0.025$, as well as a Sex × Gonadectomy interaction, $F(1,30)=16.62$, $p<0.001$. Subsequent analyses with t tests demonstrated that the stimulatory effect of amphetamine on locomotor activity was greater in intact females than in any other group ($p<0.05$). There was also a difference in the magnitude of the drug effect between the gonadectomized groups of males and females, but differences between castrated and intact male groups and between the intact male and ovariectomized females were not reliable.

By contrast, apomorphine produced a dose-dependent depression of activity in all groups, $F(1,28)=50.84$, $p<0.001$. Since the omnibus analysis also revealed main effects of Sex and Gonadectomy, $F(1,28)\geq 8.45$, both $p<0.01$, we conducted a subsequent analysis on difference scores, computed by subtracting the activity score obtained under saline treatment from that recorded under each dose of apomorphine. A mixed model analysis of these difference scores indicated that apomorphine caused greater depression of activity in the gonadectomized groups, $F(1,28)=4.37$, $p<0.05$. This influence was greater in the ovariectomized female group than in any of the other three groups ($t\geq 2.63$, $p<0.05$), which did not differ reliably. Because of the possibility of a floor effect these results should be interpreted cautiously.

DISCUSSION

The present data confirm earlier reports [3,22] of sex differences in the magnitude of amphetamine effects on locomotor activity and stereotyped behavior. In our earlier study [3] female rats displayed somewhat more intensely stereotyped behavior than males after apomorphine treatment, but the effect was not statistically significant while in the present study this sex difference was reliable. Considering the two experiments together it is clear that while the sex differences in stereotyped behavior produced by amphetamine and apomorphine are qualitatively similar, the effect is substantially more robust with amphetamine.

Previous studies of the effects of moderate doses of amphetamine reveal that this drug consistently stimulates locomotor activity [7], but the influence of apomorphine on the activity of neurologically intact animals is considerably more variable. In agreement with the present data DiChiara, Corsini, Mereu, Tissari, and Gessa [8] observed that low doses of apomorphine inhibited activity in mice. They attributed this effect to preferential activation of presynaptic dopamine receptors by apomorphine. Such an explanation would fit our data at the 0.1 mg/kg dose very nicely. Whether or not the marked suppression of activity we observed with 1 mg/kg doses of apomorphine can be explained in similar terms is an open question. Casual observations of rats given this dose taken during the activity tests indicated that most animals exhibited some degree of stereotyped sniffing and rearing which may have interfered with locomotor activity.

The present finding that ovariectomy blocked the stimulating effect of 1 mg/kg amphetamine but weakly enhanced the inhibitory effect of 0.1 and 1 mg/kg apomorphine on locomotor activity is consistent with a growing body of data suggesting that gonadal hormones can modify the behavioral effects of drugs that affect brain dopamine systems as well as several aspects of central dopamine metabolism [2, 4, 5, 6, 12, 19, 21, 29]. That ovariectomy altered the effects on activity of moderate doses of amphetamine and apomorphine without changing the magnitude of stereotyped behavior evoked by higher doses of these drugs might imply some sort of selective action of ovarian hormones on brain circuits that are activated by these dopamine agonists. It is now generally accepted that amphetamine stereotypy is dependent on the release of dopamine by nigrostriatal terminals [7]. More controversy exists about the neural systems mediating the stimulating effects on activity of lower doses of the drug, but the mesolimbic dopaminergic pathway clearly plays an important role in this effect [7, 18, 26]. Assuming this dissociation is valid, then the present data could suggest that ovarian secretions preferentially modulate the activity of the mesolimbic pathway. In fact Baum and his colleagues [2] have recently reported that estradiol, testosterone or dihydrotestosterone increase dopamine turnover in the mesolimbic but not in the nigrostriatal pathway. Moreover, autoradiographic studies indicate that the neostriatum is virtually devoid of estrogen and androgen receptors while portions of the projection field of the mesolimbic pathway contain significant numbers of target neurons for these hormones. The concentration of hormone receptors is especially high in the interstitial nucleus of the stria terminalis but significant numbers of estrogen and dihydrotestosterone target cells are also found in the olfactory tubercle, the lateral septal nucleus, and the central nucleus of the amygdala [25, 27, 28]. While the cell bodies of the dopaminergic neurons of the

nigrostriatal and mesolimbic pathways do not seem to contain receptors for steroid hormones, in parts of the interstitial nucleus of the stria terminalis cells which concentrate radioactively labeled steroid hormones are surrounded by terminals of catecholamine neurons [14,15]. Thus, there appears to be an anatomical basis for the functional distinction between the mesolimbic and nigrostriatal systems suggested by the present findings that ovariectomy selectively influences the action of dopaminergic agonists on activity.

On the other hand there is now a substantial body of evidence that indicates that gonadal hormones, especially estradiol, can modify the functioning of the nigrostriatal pathway. Following ovariectomy in rats dopamine-stimulated adenylate cyclase activity declines in both the striatum and the nucleus accumbens [19]. Ovariectomy also shortens the display of stereotyped behavior after amphetamine or apomorphine treatment in guinea pigs [24] and treatment with estradiol increases the amount of stereotyped behavior elicited by dopaminergic agonists in gonadectomized rodents of both sexes [5, 17, 24].Furthermore, estradiol treatment in castrated male rats with unilateral 6-hydroxydopamine lesions prolongs the duration of ipsilateral rotation, apparently by increasing the number of striatal dopamine receptors [16,17].

These findings imply that estrogen in some way stimulates the activity of brain dopamine systems, but other data suggest exactly the opposite action. Estrogen potentiates the catalepsy resulting from spiroperone treatment [6], reduces the changes in striatal acetylcholine induced by treatment with dopamine agonists and antagonists [10], and reduces the magnitude of apomorphine-elicited stereotyped behavior in female rats chronically pretreated with haloperidol [11].

No doubt procedural differences are partly responsible for some of these discrepancies. For example, comparing the response of ovariectomized animals and randomly cycling females as was done in the present study may provide a less sensitive test of hormonal modulation of dopamine-dependent behaviors than directly examining the effects of hormone injections in gonadectomized animals (e.g., [5, 16, 17]). However, this analysis cannot explain the failure of Baum and his colleagues to observe any effect of gonadal steroid treatment on apomorphine-elicited stereotypy in castrated male rats [2]. And while our procedure may not have been sufficiently sensitive to reveal modulating effects of ovarian secretions on stereotypy, we did observe a marked effect of ovariectomy on locomotor activity. The available evidence clearly indicates that gonadal hormones, particularly estradiol, can affect biochemical measures of both the mesolimbic and nigrostriatal systems as well as behavioral responses to drugs that affect these pathways. The present data together with the work of Baum and his colleagues suggest that hormonal influences on the functioning of dopaminergic systems may be somewhat greater in the mesolimbic pathway.

Resolution of the apparent contradictions that already exist in the literature will require careful examination of hormone effects on drug metabolism. It is already clear the female rats metabolize amphetamine more slowly than males [13,23] and estradiol increases brain levels of spiroperone [6]. Such changes may explain some of the sex differences in response to dopamine agonists and antagonists. Furthermore, the possibility that the effects of gonadal hormones on dopamine-dependent behaviors may be exerted indirectly, secondary to hormonal influences on other neurotransmitter systems that modulate midbrain dopamine neurons, should be considered. Considerable evidence exists that both GABAergic [20] and noradrenergic [1] pathways influence dopaminergic systems.

Estradiol and other gonadal steroids are known to alter several aspects of GABA metabolism [9, 12, 21, 29] and there are estradiol receptors within the cell bodies of norepinephrine neurons as well as in cells that are surrounded by noradrenergic terminals [14,15]. Considering the many possibilities for dynamic interactions among these neurochemical circuits, it is probably not surprising that a variety of apparently contradictory effects have been described.

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