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Haloperidol, But Not Apomorphine, Differentially Affects Low Response Rates of Male and Female Wistar Rats

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VAN HEST, A , F VAN HAAREN AND N E VAN DE POLL *Haloperidol, but not apomorphine, differentially affects low response rates of male and female Wistar rats* PHARMACOL BIOCHEM BEHAV **29**(3) 529–532, 1988 — Amphetamine-induced behavioral differences between male and female rats have been observed in tests for stereotyped behavior, locomotor activity and rotational behavior It has also been shown that amphetamine differentially affects schedule-controlled behavior of male and female rats The present experiments were designed to further investigate sex differences in sensitivity to agents which act upon the dopaminergic system In the first experiment, male and female rats responding on a schedule which maintained low response rates were challenged with different low (<0 5 mg/kg) doses of apomorphine Low doses of apomorphine act on the dopamine receptors on the dopaminergic terminals themselves to inhibit the release of endogeneous dopamine In the second experiment, the same subjects were given different doses of haloperidol, which selectively blocks the post-synaptic dopamine receptors The results of the present experiments showed that haloperidol, but not apomorphine, differentially affected the behavior of male and female subjects A behavioral difference between the sexes was thus observed when post-synaptic dopamine receptors were blocked by a dopamine antagonist, but not when dopaminergic stimulation of post-synaptic dopamine receptors was reduced by pre-synaptic inhibition of dopamine release These results suggest a role for post-synaptic receptor mechanisms in mediating sex differences in sensitivity to dopaminergic agents, although other possible mechanisms remain to be investigated

Apomorphine Haloperidol Behavioral differences Male and female Wistar rats

A number of experiments have shown behavioral differences between male and female rats in response to the administration of drugs which challenge the dopaminergic system. Behavioral differences between male and female rats after treatment with amphetamine (AMPH), a dopaminergic agonist, have been observed in tests for stereotyped behavior, locomotor activity and rotational behavior AMPH-induced stereotyped behavior was more intense and longer lasting in females than in males [1,2] Females showed a greater increase in locomotor activity after treatment with AMPH than did males. This effect could be blocked by ovariectomy [12]. Estradiol potentiated the AMPH-induced increase in activity when given to castrated male rats [9] Females rotated more than males when they were challenged with high doses of AMPH [11] Treatment of ovariectomized females with estradiol benzoate enhanced the rotational behavior induced by AMPH [3]. Recently, it has been shown that AMPH also differentially affects schedule-controlled behavior of male and female rats responding at low rates [13]. Response rates of both males and females on a schedule which maintained high rates of responding (differential reinforcement of high rates, DRH), decreased in a dose-dependent manner after treatment with AMPH, but differences between males and females were not observed. When rats were responding on a schedule which maintained low rates of responding (differential reinforcement of low rates, DRL) response rates increased after treatment with AMPH, but increased more for females than for males. Although it could be argued that these behavioral differences result from the well-known sex difference in drug metabolism in rats [10], other experiments have shown behavioral differences between the sexes even when brain AMPH levels are comparable [4] These experiments thus specifically suggest a modulating influence of gonadal hormones on behaviors which are thought to reflect the activity of dopaminergic neurons in the nigrostriatal and mesolimbic systems

The present experiments were designed to further investigate sex differences in sensitivity to agents which act upon the dopaminergic system Male and female rats were therefore challenged with different doses of pharmacological agents which antagonize dopaminergic functioning. In experiment 1a, the effect of different low (<0 5 mg/kg) doses of apomorphine (APO) was assessed when male and female rats were responding on a DRL 15 sec schedule of reinforcement. Low doses of APO act upon the dopamine receptors on the dopaminergic terminals themselves, to inhibit the release of endogenous dopamine [6] As a result, post-synaptic receptor activation is decreased after treatment with low doses of APO. In experiment 1b, the same subjects were challenged with different doses of haloperidol (HALO), a selective post-synaptic dopamine receptor blocking agent

METHOD

Subjects

Nine male and 9 female rats who participated in another experiment designed to measure behavioral differences between male and female rats responding on different DRL schedules [14] served as subjects All subjects were 11 months old at the start of the present experiment They were housed in group cages (3 same sex subjects to a cage) under a reversed light-dark cycle (lights on 3.30 p m –3 30 a m) Subjects were maintained on a 23-hr food deprivation schedule resulting in a deprivation to approximately 85% of free-feeding body weight [8] Body weights averaged 423 0 (±38.4) gram for males and 267 6 (±13 4) gram for females Water was always available in the homecages

Apparatus

Four standard Grason-Stadler (model 1111-L) rodent operant conditioning chambers were used Each chamber was made of Plexiglas and measured 28×30×30 5 cm The grid floor consisted of 23 stainless steel grids, spaced 1 25 cm apart Two non-retractable rodent levers (1.25 cm thick) were mounted on the intelligence panel and protruded 1 6 cm into the chamber The levers were located symmetrically to the side of the pellet retrieval unit, 8 7 cm above the grid floor Only the right lever was active in the present experiment Responses on the left lever were recorded, but had no scheduled consequences The levers required a force of at least 0 20 N to be operated Stimulus lights were mounted slightly to the side and above the levers Only the red stimulus light located next to the right lever was illuminated in the present experiment A houselight was located in the upper left hand corner of the intelligence panel Each chamber was enclosed in a Grason-Stadler research chest (model 1101) A fan was used to provide fresh air Programming of experimental contingencies and data acquisition were accomplished by means of Grason-Stadler 1200 series programming equipment, located in the experimental room itself

Procedure

Experiment 1a Preliminary training was not necessary Prior to the present experiment, all subjects had participated in another experiment in which behavior was maintained by different DRL schedules [14] Immediately following that experiment, all subjects were trained on a DRL 15 sec schedule of reinforcement On such a schedule, reinforcement (45 mg Noyes pellet) is delivered if, and only if, two successive responses are separated by at least 15 sec Sessions ended after 25 minutes or when 40 reinforcers had been obtained, whichever came first Sessions were run five days a week (Monday through Friday), during the last quarter of the subject's dark hours

Subjects received daily injections of vehicle solution (0 1% sodium metabisulfate and 0 9% NaCl in distilled water, 1 ml/kg) for 7 days prior to the first injection with APO Five different doses of APO (apomorphine HCl, freshly dissolved in vehicle solution, 0 01, 0 05, 0 10, 0.25 and 0 50 mg/kg, 1 ml/kg) were used. Subjects were treated with APO on Tuesdays and Fridays, vehicle injections were given on the other days of the week. The order of administration of APO was varied over subjects All injections were given subcutaneously (SC) in the neck region, 5 minutes prior to DRL testing

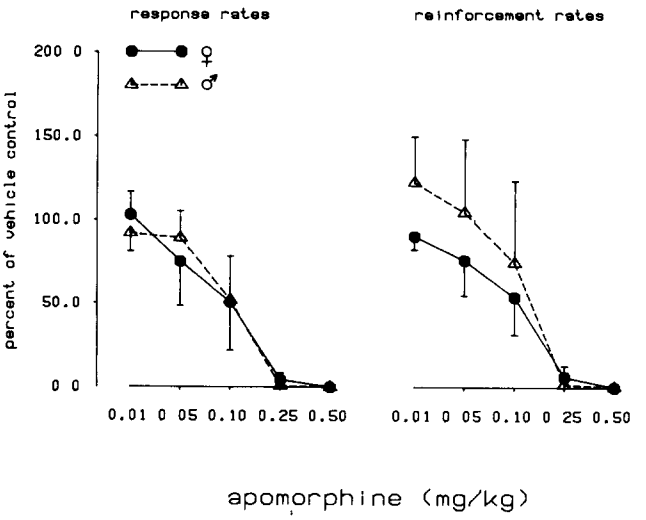

FIG 1 The effects of different doses apomorphine on response rates (left panel) and reinforcement rates (right panel) expressed as percentages (mean and standard deviation) of vehicle control values

Experiment 1b Three weeks after the completion of experiment 1a, all subjects which participated in that experiment were injected with vehicle solution (0 5% lactic acid and 0 9% NaCl in distilled water, 1 ml/kg) for 6 days prior to the first injection with HALO Five different doses of HALO (haloperidol dissolved in vehicle solution, 0 01, 0 05, 0 10, 0 25 and 0 50 mg/kg, 1 ml/kg) were used Subjects were treated with HALO on Tuesdays and Fridays, vehicle injections were given on the other days of the week The order of administration of HALO was varied over subjects All injections were given subcutaneously (SC) in the neck region, 25 minutes prior to DRL testing

RESULTS

Experiment 1a

Figure 1 shows the effects of increasing doses of APO on response rates (responses/minute) and reinforcement rates (obtained reinforcers/minute) of male and female rats exposed to a DRL 15 sec schedule of reinforcement A running average over 7 sessions, starting 7 days before the first APO administration and continuing through the vehicle sessions interspersed between drug sessions, was calculated to obtain vehicle control values Pre-drug vehicle response rates were significantly higher for males than for females [4 54 and 3 79 responses/minute respectively, Student's $t(16)=2\ 91, p<0.01$] Differences in reinforcement rate were not observed [males 1 90, females· 2 36 reinforcers/minute, $t(16)=2\ 06, p>0.05$]

Drug effects on response rates and reinforcement rates of males and females were expressed as the percentage change from vehicle control values for males and females separately Analysis of variance with the main factors Sex and Doses (repeated measures) showed a dose-dependent decrease in the response rates, $F(4,64)=145.44$, and reinforcement rates, $F(4,64)=99\ 33$, both $p<0\ 01$, after administration of different doses of APO. Overall sex differences in response rates, $F(1,16)=0\ 002$, and reinforcement rates, $F(1,16)=3\ 92$, both $p>0\ 5$, expressed as percentage of vehicle control values were not observed A significant Sex by

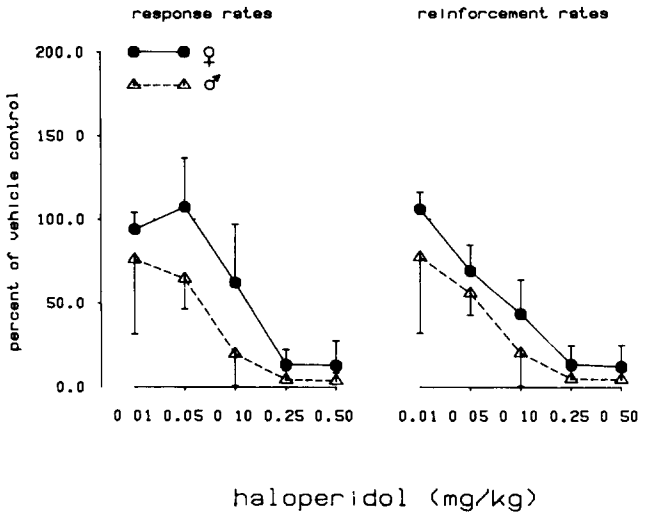

FIG 2 The effects of different doses haloperidol on response rates (left panel) and reinforcement rates (right panel) expressed as percentages (mean and standard deviation) of vehicle control values

Doses interaction was not observed for response rates, $F(4,64)=1\ 53$, $p>0\ 2$ Treatment with increasing doses of APO therefore decreased the response rates of males and females to the same degree Reinforcement rates of females were affected by lower doses of APO than those of males, $F(4,64)=2\ 91$, $p<0\ 05$

Experiment 1b

Figure 2 shows the effects of increasing doses of HALO on the behavior of male and female rats exposed to a DRL 15 sec schedule of reinforcement. Response rates and reinforcement rates were calculated and analysed as in experiment 1a. Pre-drug response rates were higher in males (3 92 responses/minute) as compared to females [3 32 responses/minute, $t(16)=2\ 58$, $p<0\ 05$] Differences in the number of reinforcers per minute were not observed [reinforcement rate, males 2 41, females 2 60, $t(16)=1\ 19$, $p>0\ 02$]

Response rates, $F(4,64)=59\ 79$, $p<0\ 01$, and reinforcement rates, $F(4,64)=65\ 42$, $p<0\ 01$, decreased dose-dependently after administration of different doses of HALO Males were more sensitive to the inhibitory effects of HALO than females response rates of females after administration of different doses of HALO were higher than those of males, $F(1,16)=16\ 35$, $p<0\ 001$ Females also obtained more reinforcers per minute, $F(1,16)=13\ 53$, $p<0\ 001$ A significant Sex by Doses interaction effect was observed for response rates, $F(4,64)=2\ 99$, $p<0\ 05$ Post-hoc comparisons between the sexes showed that response rates of females were higher than those of males after they were challenged with intermediate doses of HALO [Student's t-test, 0 05 mg/kg $t(16)=3\ 70$, $p<0.01$, 0 10 mg/kg $t(16)=3\ 24$, $p<0.01$, 0 25 mg/kg $t(16)=2.54$, $p<0\ 05$, two-tailed test of significance], but not after treatment with either the lowest, $t(16)=1\ 19$, $p=0.25$, or the highest dose, $t(16)=1\ 81$, $p=0\ 09$ Other significant Sex by Doses interactions were not observed [reinforcement rates $F(4,64)=1\ 10$, $p>0\ 3$]

DISCUSSION

Male and female Wistar rats responded at low rates when treated with either low doses of APO or HALO APO in low doses impedes DA transmission and reduces the stimulation of post-synaptic dopamine receptors by endogenous DA [6] Response rates decreased dose-dependently after treatment with APO, but differences between the sexes were not observed. These results confirm earlier findings which also showed that spontaneous shuttling activity decreased both for males and females after administration of low doses of APO [12]. Sex differences in sensitivity to APO have thus far only been observed when rats were challenged with post-synaptically acting moderate to high doses of APO [12]

HALO is a selective dopamine blocker, acting on the post-synaptic dopamine receptor The results of the present experiment showed that low-frequency response rates decreased dose-dependently after administration of different doses of HALO, while males were more sensitive to the inhibitory effects of HALO than females As such, they confirm previous observations in which it was shown that HALO administration affected locomotor activity [1] and cocaine self-administration [5] in a sex-dependent manner

In the present experiment, behavioral differences between the sexes were only observed after rats were challenged with HALO, but not after APO administration. These observations could be due to a number of different variables First of all, it could be argued that the results of the present experiment combined with the observed behavioral differences between the sexes when rats are challenged with high, post-synaptically acting doses of APO [12] or AMPH [13] obviously implies sex differences in post-synaptic dopamine receptor mechanisms Since a number of experiments have shown that the functional activity of the dopaminergic system may be altered by estrogen administration to male and female rats [3,7], it could be argued that the functional activity of the post-synaptic DA receptor might be influenced by female gonadal hormones The absence of a differential behavioral effect after low doses of APO might suggest that sex differences only exist in DA receptor fields which are primarily innervated by DA neurons which lack DA autoreceptors However, such a suggestion obviously needs to be confirmed by more direct assessment of the influence of gonadal hormones on direct measures of DA receptor sensitivity in both sexes

Since decreased post-synaptic DA receptor activation due to the reduced availability of endogeneous dopamine did not lead to behavioral differences between the sexes other possible mechanisms than post-synaptic DA receptor sensitivity need to be entertained It could be the case that sex differences exist in APO and/or HALO metabolism, even though, to our knowledge, such differences have not yet been reported If differences in APO metabolism exist, it might be the case that differences in DA availability are masked by differences in post-synaptic receptor sensitivity Similarly, differences in HALO metabolism may lead to differences in DA receptor blocking which may have produced the observed behavioral differences between the sexes However, other possible mechanisms must also be considered since behavioral differences in response to the administration of the DA agonist AMPH have also been observed when drug brain levels were comparable for both sexes [4]

In summary, a number of studies have shown behavioral differences between the sexes after administration of drugs which interfere with dopaminergic transmission Some studies have suggested an important role for the female gonadal hormone estrogen in mediating these differences

Most studies also have suggested an important role for postsynaptic DA receptor mechanisms in mediating these differences, but definitive interpretations regarding these differences may only be expected after exhaustive neuropharmacological and neurobehavioral studies have been conducted

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