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Increased Investigative Activity After Presynaptic Dopaminergic Stimulation Measured by a Fixed-Interval Recording Procedure

A. U. HÖGLUND AND B. J. MEYERSON

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

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HÖGLUND, A. U. AND B. J. MEYERSON. *Increased investigative activity after presynaptic dopaminergic stimulation measured by a fixed-interval recording procedure.* PHARMACOL BIOCHEM BEHAV 22(3) 403–406, 1985.—A fixed-interval recording procedure was used to study the effects of catecholaminergic agonists and antagonists on the exploratory behaviour pattern in male rats. One particular element of this pattern, the investigative behaviour, was found to be sensitive to drugs acting on both noradrenaline and dopamine receptors. An increase in investigative activity (IA) and a slight decrease in the other exploratory (OE) activity was caused by α-methyl-p-tyrosine. The α_2-receptor agonists clonidine and B-HT 933 decreased both the IA and the OE activity. These effects were counteracted by yohimbine. Activation of presynaptic dopamine receptors by low doses of apomorphine (≤0.2 mg/kg) and B-HT 920 resulted in significant increase in IA and a decrease in OE activity. Higher doses of apomorphine (0.7 and 1.0 mg/kg) decreased or abolished IA but increased OE activity. We assume this effect to be due to postsynaptic receptor stimulation. Pretreatment with haloperidol counteracted the effects of apomorphine and B-HT 920. An increase in IA is probably due to a decrease in dopaminergic activity, which can be induced by presynaptic receptor activation. The decrease in IA after presynaptic noradrenergic stimulation cannot at present, however, be related to a specific influence, since the possibility of general effects on locomotion cannot be ruled out. The increase in IA observed after presynaptic dopaminergic activation and the fixed-interval recording procedure may be valuable instruments in the search for selectively acting compounds with dopaminergic activity.

Exploratory behaviour Habituation Dopamine Noradrenaline α-Methyl-p-tyrosine Yohimbine Clonidine B-HT 920 Haloperidol Apomorphine

A multivariate behavioural method for the study of drug action on central nervous processes was presented in a previous report [7]. Using this method, several exploratory and socio-sexual elements of behaviour in the laboratory rat were defined and recorded in terms of duration, frequency and latency. The studies were made by direct observation during a defined time interval. From the various behaviour elements and variables, a "behavioural profile," characteristic for a particular stimulus situation, was established.

Among the different elements of behaviour displayed during the exploratory test situation, there is one of particular interest, which we have called "investigating." If the animal is not very well acquainted with the observation situation this behaviour has a short duration, low frequency and long latency. This contrasts with other elements connected with the exploratory act, such as "sniffing" and "rearing," which in a novel situation have a long duration, high frequency and short latency. We found in a previous study [5] that habituation to the experimental situation resulted in an increase in the duration and frequency and a decrease in the latency of the investigative behaviour. Impairment of monoaminergic biosynthesis resulted in similar changes [6].

The purpose of the present experiments was to determine whether catecholaminergic agonists and antagonists could influence this particular element of behaviour.

We have found that when one or a few elements of a behavioural pattern are of particular interest, the observation can be simplified by a fixed-interval recording procedure, which is presented in this article.

The results showed that the investigative behaviour is sensitive both to drugs acting on noradrenaline receptors and to those with an effect on dopamine receptors. The most distinct finding was an increase in investigative activity after presynaptic dopamine receptor activation.

METHOD

Animals and Laboratory Conditions

Male Sprague-Dawley rats (purchased from Anticimex, Sollentuna, Sweden) weighing 350–400 g were housed in steel cages (300×200×500 mm) with five animals in each. They were provided with commercial rat pellets (Ewos R3) and tap water ad lib. The temperature was kept at 21±1°C. The day-night cycle was reversed with light between 21.00 hr

TABLE 1

THE EFFECTS OF CATECHOLAMINERGIC AGONISTS AND ANTAGONISTS ON THE INVESTIGATIVE ACTVITY AND OTHER EXPLORATORY ACTIVITY (OE-ACT)

Drug Treatment	Dose mg/kg	Investigative ACT. DUR.	FRE.	LAT.	OE-ACT. DUR.	N
a. Saline		9.2	3.0	310.4	126.7	36
b. α-MT	100.0	14.7†	5.5†	188.7	121.3†	12
c. Yohimbine	1.0	11.6*	5.0†	306.0	126.6	12
d. Clonidine	0.02	1.6†	1.0†	243.3	118.1‡	12
+Yohimbine	1.0	8.2	5.0	244.3	130.7	12
e. B-HT 933	5.0	0.0‡	0.0‡	900.0†	40.2‡	12
+Yohimbine	1.0	18.9‡	7.5†	123.9†	121.8‡	12

Treatment regimen (time prior to test): α-methyl-p-tyrosine (α-MT) IP 4 hr, yohimbine IP 30 min, clonidine SC and B-HT 933 IP 15 min. Median values are given. Durations (DUR). and latencies (LAT.) are given in seconds and frequencies (FRE.) in scores. N represents number of animals. The Mann-Whitney U-test was used to test the differences between drug-treated and saline-treated groups. Significances are denoted *$p<0.05$, †$p<0.02$, ‡$p<0.001$.

and 09.00 hr. All tests were performed between 12.00 hr and 16.00 hr under conditions of dimmed light.

The Exploratory Behaviour Test

A fixed-interval recording method for scoring exploratory behaviour was used. Six animals were transferred from their home cages to separate observation boxes, one animal per box (plywood boxes, 400×600×400 mm, each with a Plexiglas front and a floor covered with wood-shavings). A total of 204 animals were used in two experiments (one original and one replicate). The animals were divided into 34 groups with 6 animals in each. Some animals were given two active substances. In these cases, the first treatment was a low dose of a short acting compound and the treatment interval was at least one week. Each animal was observed for 10 sec each minute over a total period of 15 minutes. During this period the duration (total time for which a behaviour was performed, frequency (the number of times a behaviour was performed) and latency (the time from the start of recording to the first occurrence of a behaviour) of the displayed behaviour elements were scored in six animals simultaneously. The elements of behaviour recorded are reported in detail elsewhere [7] but are described below.

Sniffing. Rapid movements of whiskers while the subject explores. Scores were taken regardless of whether the animals were sniffing while moving or sniffing while stationary.

Rearing. Standing on hind legs while sniffing.

Investigating. Intense sniffing directed at a particular object such as a fecal bolus, wood-shaving etc., which is picked up by the animal and explored.

Other elements of behaviour observed were intense sniffing, grooming, freezing, resting, and scanning. The scores were low, however, and these data will not be presented. An increase in the duration and frequency of the investigative behaviour and a decrease in latency will be referred to in the following as "increased investigative activity" (increased IA). The durations of sniffing and rearing were added together and denoted "other exploratory activity" (OE activity).

Drugs

The following drugs were used: DL-α-methyl-p-tyrosine-methylester HCl (Hässle), apomorphine HCl (Sandoz), clonidine HCl (Boehringer-Ingelheim), B-HT 920 (Boehringer-Ingelheim), B-HT 933 (Boehringer-Ingelheim), yohimbine (Sigma), and haloperidol (Leo). Haloperidol was dissolved in a few drops of glacial acetic acid and saline was added to volume. The other substances were dissolved in saline only. Doses refer to the salts referred to.

RESULTS

The frequency of sniffing and rearing always changed in the same direction as the duration and did not provide any further information, nor did the latencies of these behaviours, which remained short throughout in the different treatment categories.

The tyrosine-hydroxylase inhibitor α-methyl-p-tyrosine (α-MT, 100 mg/kg intraperitoneally (IP) administered 4 hr before the test) caused an increase in IA, but slightly decreased OE activity (Table 1b). In contrast, the α-receptor agonist [1] clonidine (0.02 mg/kg given subcutaneously (SC) 15 min before the test; Table 1d) decreased IA. This effect was counteracted by the α-receptor blocking agent [1] yohimbine (1.0 mg/kg IP 30 min prior to test). The substance B-HT 933 (5.0 mg/kg IP 15 min before the test), which has α_2-adrenoreceptor agonistic effects [3], also decreased IA. The effect of B-HT 933 was reversed by yohimbine (1.0 mg/kg, Table 1e). In fact, combined treatment with yohimbine and B-HT 933 resulted in increased IA. Both clonidine and B-HT 933 diminished OE activity. These effects were counteracted by yohimbine.

Activation of presynaptic dopamine receptors by apomorphine (doses $\leq$0.5 mg/kg given SC 15 min before the test) enhanced IA and decreased OE activity (Table 2e). Higher doses ($\geq$0.7 mg/kg) caused an abolition of IA and an increase in OE activity as compared with control animals.

IA was also accentuated after administration of the specific dopamine autoreceptor activating substance [3] B-HT 920 (IP) 15 min before the test; Table 2f). Simultaneously OE activity decreased significantly.

TABLE 2

THE EFFECTS OF DOPAMINERGIC AGONISTS AND ANTAGONISTS ON THE INVESTIGATIVE ACTIVITY AND OTHER EXPLORATORY ACTIVITY (OE-ACT)

Drug Treatment	Dose mg/kg	Investigative ACT. DUR.	FRE.	LAT.	OE-ACT. DUR.	N
a. Saline		10.0	3.5	306.5	127.3	36
b. Haloperidol	0.5	0.5‡	1.0†	92.0‡	98.9‡	12
c. Apomorphine	0.2	25.1†	9.0†	181.7*	112.2†	12
+Haloperidol	0.5	5.8	3.0	121.6‡	55.1‡	12
d. B-HT 920	0.03	28.9†	11.0†	60.7‡	93.1†	12
+Haloperidol	0.5	7.7	2.0	182.8*	74.7	12
e. Apomorphine	0.03	13.2†	5.0*	125.2*	123.4*	12
	0.1	20.4‡	8.0‡	154.3†	115.8‡	12
	0.2	23.9†	7.0†	157.0*	114.2†	12
	0.5	15.1†	7.5‡	124.9*	116.2*	12
	0.7	0.0‡	0.0‡	900.0‡	144.9‡	12
	1.0	0.0‡	0.0‡	900.0‡	144.1‡	12
f. B-HT 920	0.003	9.4	4.5	218.0	129.4	12
	0.01	11.3	5.5	363.4	127.5	12
	0.03	24.0†	9.0†	61.4‡	103.9†	12
	0.1	54.0†	12.5‡	62.4‡	45.5‡	12

Substances were injected SC 15 min before the test except for haloperidol (1.5 hr, IP). For explanation of abbreviations, see Table 1.

Haloperidol (0.5 mg/kg IP 1.5 hr before the test; Table 2b), which has dopamine receptor blocking properties [2], almost abolished IA, while OE activity decreased relatively less. Haloperidol counteracted the effects of both apomorphine and B-HT 920 (Table 2c and d).

DISCUSSION

The use of compounds with effects on the central nervous system in behavioural studies may have two alternative objectives. Either the drugs may be administered as a tool to investigate neurophysiological events involved in a particular behaviour, or a behaviour element may be utilized as a test of drug action. In the present study the former alternative applied, the aim being to study the IA further by the use of catecholaminergic agonists and antagonists, in an attempt to assess which of the catecholaminergic transmitters are responsible for the previously observed changes in IA in response to different treatments [6].

In concordance with our previous study [6] the α-MT treatment caused an increased IA. As this substance is known to inhibit the synthesis of both NA and DA this finding must be regarded as an indication of an involvement of catecholaminergic mechanisms in the regulation of the IA. The other substances were used to distinguish between these transmitters.

The noradrenergic agonists clonidine and B-HT 933 effectively reduced the OE activity and to a greater extent the IA. Clonidine in a dose of 0.02 mg/kg acts predominantly on presynaptic α_2-receptors [1]. B-HT 933 has also been found to exert α_2-receptor agonistic properties [3]. Stimulation of presynaptic α-adrenoreceptor results in a decrease in the utilization of noradrenaline, which is presumably reflected in diminished activity in postsynaptic transmitter events. We therefore suggest that noradrenergic activity is necessary for the occurrence of IA. This suggestion is further supported by the fact that the clonidine effect was reversed by yohimbine, IA being restored to normal. We cannot at present exclude the possibility of unspecific effects of these agents, since the effect on the OE activity indicates a general decline in the activity of the animal. A comparison between the results of the combined treatment with clonidine and yohimbine, on the one hand, and B-HT 933 and yohimbine, on the other, reveals that B-HT 933 has some other effects besides presynaptic noradrenergic stimulation. This is suggested by the increase in IA after treatment with B-HT 933 and yohimbine combined. This additional effect may be masked by the α_2-noradrenergic stimulation, but is reflected in the IA response on blocking of the α_2-receptor stimulation by the antagonist yohimbine. With the present knowledge, however, this effect cannot be attributed to a particular transmitter.

The dopamine agonistic property of apomorphine is dose-dependent. Observations have indicated that lower doses ($\leq$0.2 mg/kg) act presynaptically, and higher doses postsynaptically [8]. B-HT 920 was recently found to activate dopamine autoreceptors [3]. In the present study these two substances (apomorphine, low dose, and B-HT 920) caused a drastic increase in IA, suggesting that dopamine may normally have a tonic-inhibitory effect on IA, which is eliminated by the autoreceptor activation. The fact that the dopamine receptor antagonist haloperidol counteracted the increase in IA induced by the presynaptic agonist further support this possibility.

It has, however, been proposed that the action of haloperidol on the dopamine system is exerted on both pre- and postsynaptic dopamine receptors [2,10]. This might explain why haloperidol reduced both the IA and OE activity when injected alone. There is also some evidence that haloperidol can cause an α_1-receptor blockade [4]. It is doubtful, how-

ever, whether such blockade would result in diminished locomotor activity.

Higher doses of apomorphine decreased or completely abolished IA, presumably by enhancing postsynaptic dopaminergic activity. The increase in sniffing and rearing (OE activity) reflects an increase in the stereotype activity which can be expected at these dose levels of apomorphine [8]. Interestingly, the compound B-HT 920 did not follow the bimodal dose-response relationship which characterizes apomorphine. We consider the reason for this to be that B-HT 920 lacks postsynaptic dopaminergic agonistic properties. The functional observations in this study are clearly in agreement with available biochemical data [3].

We conclude that IA is sensitive to drugs acting on noradrenaline as well as dopamine receptors. An increase in IA is probably due to a decrease in dopaminergic activity, which can be induced by presynaptic receptor activation. The decrease in IA after presynaptic noradrenergic stimulation cannot at present, however, be related to a specific influence, since the possibility of general effects on locomotion cannot be ruled out.

The results may also be looked upon from the behavior-physiological point of view. We have previously suggested a relationship between IA and habituation [5]. IA was increased after repeated exposure to the testing arena. The findings in the present study are in line with the concept of a tonic dopaminergic inhibitory influence on IA. On this basis we may assume that dopamine also is implicated in the process of habituation.

The fixed-interval recording of investigative behaviour would seem to be a useful instrument in the search for selectively acting compounds with dopaminergic activity.

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REFERENCES

1. Andén, N.-E., M. Grabowska and U. Strömbom. Different alpha-adrenoreceptors in the central nervous system mediating biochemical and functional effects of clonidine and receptor blocking agents. *Naunyn Schmiedebergs Arch Pharmacol* **292:** 43–52, 1976.
2. Andén, N.-E. and M. Grabowska-Andén. Presynaptic and postsynaptic effects of dopamine receptor blocking agents. In: *Advances in Neurology,* vol 24, edited by L. J. Poirier, T. L. Sourkes and P. J. Bédard. New York: Raven Press, 1979, pp. 235–244.
3. Andén, N.-E., H. Nilson, E. Ros and U. Thornström. Effects of B-HT 920 and B-HT 933 on dopamine and noradrenaline autoreceptors in the rat brain. *Acta Pharmacol Toxicol (Copenh)* **52:** 51–56, 1983.
4. Brannan, M. D., J. J. Riggs, W. E. Hageman and T. P. Pruss. A comparison of the cardiovascular effect of haloperidol, thioridazine and chlorpromazine HCl. *Arch Int Pharmacodyn* **244:** 48–57, 1980.
5. Höglund, A. U. and B. J. Meyerson. Effects of lysine vasopressin in an exploratory behaviour test situation. *Physiol Behav* **29:** 189–193, 1982.
6. Höglund, A. U. and B. J. Meyerson. Facilitatory effects of monoamine synthesis inhibitors on lysine-vasopressin induced changes in the exploratory behaviour pattern of male rats. *Physiol Behav,* submitted.
7. Meyerson, B. J. and A. U. Höglund. Exploratory and sociosexual behaviour in the laboratory rat. *Acta Pharmacol Toxicol (Copenh)* **48:** 168–180, 1981.
8. Strömbom, U. Catecholamine receptor agonists. Effects on motor activity and rate of tyrosine hydroxylation in mouse brain. *Naunyn Schmiedebergs Arch Pharmacol* **292:** 167–179, 1976.
9. Strömbom, U. Antagonism by haloperidol of locomotor depression induced by small doses of apomorphine *J Neural Transm* **40:** 191–194, 1977.
10. Walters, J. R. and R. H. Roth. Dopaminergic neurons: An in vivo system for measuring drug interactions with presynaptic receptors. *Naunyn Schmiedebergs Arch Pharmacol* **296:** 5–14, 1976.