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Dopamine Mediated Behavior and GABA Influence

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WESTERLING, P., S. LINDGREN AND U. HÖGLUND. *Dopamine mediated behavior and GABA influence.* PHARMACOL BIOCHEM BEHAV **31**(3) 593–596, 1988.—The possibility of interactions between GABA and dopaminergic central nervous mechanisms in the expression of spontaneous behavior was investigated using the behavior pattern shown by male rats in an exploratory test situation. The present study corroborates the facilitatory action of low doses of the dopamine agonist apomorphine on the investigative activity element of the male rats exploratory behavior pattern, as shown previously. In addition it was found that the GABA agonist baclofen in different doses (1.2–4.8 mg/kg IP) selectively increased this activity. Pretreatment with a submaximal dose of baclofen (1.2 mg/kg) potentiated the effect of apomorphine (25 μg/kg), indicating that baclofen has the same effect on behavior as presynaptically active dopamine agonists. Thus GABA mechanisms might influence dopamine mediated behavior. The investigative activity which previously has been considered to reflect the adaptive state of an animal is suggested to be influenced by a changed activity in GABA neurons, DA neurons or an interaction between these two systems in the various brain structures involved in the expression of this behavior.

Behavior Dopamine GABA Apomorphine Baclofen

DURING the past years we have established a behavioral model comprising elements of exploratory behavior of which some have been found to be sensitive to changes in dopamine activity (2). In an unfamiliar environment the laboratory rat displays several elements of behavior. Some of these have a short latency to onset, for instance rearing, ambulation and sniffing. Other behaviors appearing later during the test session involve more self-directed and item-specific behaviors. One behavior, which belongs to this latter category, is the behavioral element we define as investigating. The occurrence of this behavior is considered to be related to the adaptive state of the animal (2). The investigating behavior has been found to increase after treatment with dopamine agonists acting at presynaptic receptors (3), suggesting that dopamine neurons exert an inhibitory influence on the investigative activity which can be abolished by stimulation of dopamine autoreceptors.

In the present study we have adopted the hypothesis that the nigra-striatal dopamine pathway is involved in the regulation of this behavior and that the descending striato-nigral gamma-aminobutyric acid (GABA) pathway is of importance. The specific aim was to examine the functional relationship between GABA and dopamine activity in the control of exploratory behavior and to get further information about the transmitter mechanisms involved in adaptive processes.

METHOD

Approximately 85 male Sprague-Dawley rats (specific pathogen-free, purchased from Anticimex, Sollentuna, Sweden) weighing between 350–400 g were used. They were kept under a reversed day-night cycle (12/12 hr) with the light off between 9.00 a.m. and 9.00 p.m. and fed commercial food pellets and tap water ad lib. The animals were housed two in each cage in an air-conditioned room with the temperature kept at 21±1°C and the humidity controlled.

The rats were conditioned to the laboratory routines and handling before being taken into the experiments. The conditioning consisted of six encounters with the test arena (for 10 min each time) during two weeks before the experiments began.

Experimental Procedure

Observations were done during the dark phase of the light cycle. The animal was transferred from its home cage to an observation arena (40×60×40 cm). The floor was covered with wood shavings which previously had been exposed to other animals. Behavioral recordings started immediately after the animal had been placed in the observation cage, and lasted for ten minutes. Recordings were performed by direct observation under dimmed light conditions. The duration (total time) for which a behavior was performed was recorded by means of a computer for the following behaviors:

Rearing—standing on the hind-legs.
Sniffing—moving whiskers while exploring.
Intense sniffing—sniffing directed at a particular object.
Investigating—an object such as a fecal bulb, wood-shaving, etc., is picked up and further explored.
Resting—lying down.
Scanning—moving the head from side to side.
Grooming—licking and/or nibbling the fur.

For further descriptions of the behavioral test see (5).

TABLE 1

EFFECTS OF BACLOFEN ON THE EXPLORATORY BEHAVIOR IN MALE RATS

Treatment Dose, mg/kg	Total Time sec	Duration (sec) Rearing	Sniffing	Intense Sniffing	Investigating
Untreated	436	180 ± 14	222 ± 12	24 ± 2	10 ± 3
0.3	437	169 ± 11	200 ± 9	34 ± 8	34 ± 11
0.6	374	141 ± 49	174 ± 13†	39 ± 4*	20 ± 6
1.2	380	96 ± 17*	186 ± 14*	39 ± 4*	59 ± 9†
2.4	356	52 ± 17‡	141 ± 15‡	40 ± 6*	123 ± 26‡
4.8	74	0 ± 0‡	9 ± 1‡	1 ± 0†	64 ± 4†

The rats were injected with baclofen intraperitoneally, 30 minutes prior to testing. The values are means ± S.E.M. with 6–8 animals per group (baclofen 4.8 mg/kg, n=4). The statistical significances of the difference from the untreated group were calculated using one-way Anova followed by Student's *t*-test (*$p<0.05$, †$p<0.01$, ‡$p<0.001$). Total time means total time performing the four behaviors during a 600 sec test.

TABLE 2

EFFECTS OF APOMORPHINE ON THE EXPLORATORY BEHAVIOR IN MALE RATS

Treatment Dose, μg/kg	Total Time sec	Duration (sec) Rearing	Sniffing	Intense Sniffing	Investigating
Untreated	438	165 ± 9	223 ± 10	36 ± 7	14 ± 5
25	397	159 ± 11	177 ± 7	23 ± 6	38 ± 16
50	414	99 ± 7†	205 ± 15	47 ± 9	63 ± 22*
100	410	77 ± 12‡	221 ± 7	37 ± 8	75 ± 14†
200	339	18 ± 13‡§	250 ± 25	17 ± 4*§	54 ± 15*
400	308	20 ± 20‡§	270 ± 27	1 ± 0‡#	17 ± 6¶

The rats were injected with apomorphine subcutaneously, 10 minutes prior to testing. The values are means ± S.E.M. (n=6–8). Statistical calculations as in Table 1 for comparison with untreated controls. The statistical significances for the difference between apomorphine treatment 100 μg/kg and the two groups with higher doses are indicated (§$p<0.05$, ¶$p<0.01$, #$p<0.001$). Total time means total time performing the four behaviors during a 600 sec test.

Experimental Schedule

The animals were randomly distributed into two groups. One group received different doses of baclofen (0.3, 0.6, 1.2, 2.4 or 4.8 mg/kg IP, Ciba-Geigy) dissolved by means of ultrasound and heating in saline. The second group received different doses of apomorphine HCl (25, 50, 100, 200 or 400 μg/kg SC, Sandoz) dissolved in saline. Two weeks after the apomorphine treatment this group was given baclofen (1.2 mg/kg, IP) in combination with different doses of apomorphine (25, 100 or 200 μg/kg SC). Baclofen was given 30 min and apomorphine 10 min before the experimental trial.

Statistical Methods

Statistical significances of the differences between groups were calculated using one-way analysis of variance (ANOVA) followed by *t*-tests. The results were expressed as means±S.E.M. and the different levels of significance used were $p<0.05$, $p<0.01$, and $p<0.001$.

RESULTS

The tested drugs did not produce any significant effects on scanning and grooming behaviors (data not shown). The highest dose of baclofen increased resting which is reflected in the short total time of exploratory activity (Table 1). Except for this, data regarding these behaviors will not be considered.

Increasing doses of baclofen (0.6–2.4 mg/kg) increased the investigating and intense sniffing and decreased the rearing and sniffing activities. At the highest dose of baclofen (4.8 mg/kg) the rearing, sniffing and intense sniffing elements of behavior were almost abolished, whereas the time devoted to the investigative activity was still increased over untreated animals (Table 1).

Increasing doses of apomorphine (25–100 μg/kg) gave no significant changes in the sniffing and intense sniffing activities (Table 2). However, a decreased rearing activity was observed with a concomitant increase in the investigative activity. Doses of 200 and 400 μg/kg decreased the rearing, intense sniffing and investigative activities in comparison to what was observed after 100 μg/kg. At these doses the sniffing activity tended to increase, however not significantly.

A submaximal dose of baclofen (1.2 mg/kg) with regard to the effects on the investigating behavior was combined with different doses of apomorphine (25, 100 or 200 μg/kg) in order to explore a possible interaction between the effects of the two drugs (Table 3). Apomorphine further decreased the rearing activity seen after the baclofen treatment. Following the lowest dose of apomorphine the effects on the investigative activity significantly exceeded what was produced when the agents were given alone ($p<0.05$ for baclofen 1.2 mg/kg versus baclofen 1.2 mg/kg + apomorphine 25 μg/kg and $p<0.001$ for apomorphine 25 μg/kg versus baclofen 1.2 mg/kg + apomorphine 25 μg/kg).

In contrast to the lowest dose of apomorphine, the highest dose (200 μg/kg) of apomorphine given with baclofen significantly increased the sniffing and decreased the investigative activity. See also Fig. 1.

DISCUSSION

In accordance with previous findings, apomorphine in low doses increased the investigating and decreased the rearing activities (3). This effect is probably the result of a presynaptic dopamine agonistic effect of apomorphine since an analogous effect was obtained after treatment with a more selectively acting presynaptic dopamine agonist, B-HT 920 (3).

In addition to a dopamine involvement, the present investigation demonstrates an influence of GABA. The GABA-B-receptor agonist baclofen had a similar effect on the investigative activity as low doses of apomorphine. Other studies in our laboratory have shown that both GABA-A-receptor stimulation (by muscimol) and GABA transaminase inhibition (by ethanolamine O-sulphate) modified exploratory behavior in the same manner as B-HT 920 (Westerling *et al.* in manuscript). Thus, the investigating behavior seems to be increased following GABA-A- and/or GABA-B-receptor stimulation.

TABLE 3

EFFECTS OF APOMORPHINE ON THE EXPLORATORY BEHAVIOR OF MALE RATS PRETREATED WITH BACLOFEN

Treatment Dose	Total Time sec	Duration (sec) Rearing	Sniffing	Intense Sniffing	Investigating
Untreated	479	148 ± 11*	296 ± 16‡	23 ± 2†	12 ± 3†
Baclofen (1.2 mg/kg)	380	96 ± 17	186 ± 14	39 ± 4	59 ± 9
+ Apomorphine (25 μg/kg)	378	39 ± 7‡	200 ± 13	23 ± 6†	116 ± 15‡
+ Apomorphine (100 μg/kg)	236	1 ± 0‡	161 ± 16	9 ± 3‡	65 ± 12
+ Apomorphine (200 μg/kg)	297	0 ± 0‡	289 ± 39†	4 ± 1‡	4 ± 3‡

The rats were injected with apomorphine subcutaneously and baclofen (1.2 mg/kg) intraperitoneally 10 and 30 minutes prior to testing, respectively. The values are means ± S.E.M. (n=6–8). The statistical significance from the baclofen-treated group was calculated using one-way Anova followed by Student's *t*-test (*$p<0.05$, †$p<0.01$, ‡$p<0.001$). Total time means total time performing the four behaviors during a 600 sec test.

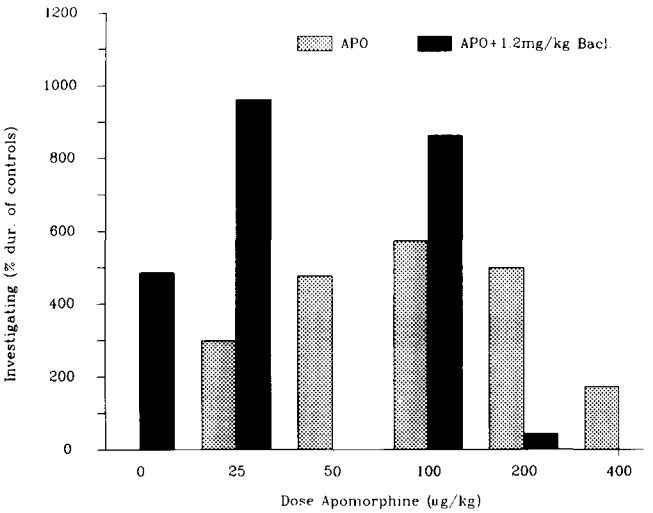

FIG. 1. The figure shows the effect of different doses of apomorphine alone and in combination with 1.2 mg/kg baclofen on the percentual duration of the investigative activity out of the total exploratory time.

Previous studies have shown interactions between nigro-striatal and striato-nigral dopamine-GABA pathways (6,7). Moreover, local injection of GABA agonists into the entopeduncular, subthalamic nuclei or substantia nigra reticulata caused a behavioral syndrome very similar to what was observed after peripheral injections of amphetamine or apomorphine (7,8). According to Scheel-Krüger's hypothesis, which is based on behavioral, electrophysiological and biochemical data, dopamine receptor stimulation in the striatum activates the descending striato-entopeduncular, striato-nigral and pallido-subthalamic GABA pathways (9) and consequently induces a GABA mediated inhibition of efferent neurons localized in these nuclei.

Low doses of apomorphine activate presynaptic dopamine receptors (10,11); this could result in a decreased dopamine activity leading to a decreased activity in the descending GABA pathways. It is suggested that nigral GABA efferents are disinhibited by the apomorphine treatment.

This suggestion leads further to the question of where baclofen exerts its action to increase the investigative activity. One possible site is the thalamus which recently has been shown to be reached by GABA fibers originating in the substantia nigra (4). Thalamus has direct anatomical links with the motor cortex, superior colliculus, corpus striatum and cerebellum. Therefore the thalamus is ideally situated to coordinate tectal, cerebellar and basal ganglia influences on locomotor behavior. Injections of GABA agonists into the thalamus have been found to produce immobility and catalepsy (1). This is in agreement with our finding where the highest dose of baclofen almost abolished the locomotor activity. It is, however, important to note that although the rearing and sniffing activities were reduced, a fairly high investigative activity was maintained.

When apomorphine was given alone an inverted U dose-response relationship was observed with regard to the investigative activity. Doses higher than 400 μg/kg of apomorphine lead to depression of investigating accompanied with stereotypic sniffing (data not shown), probably due to postsynaptic receptor stimulation (10,11).

In addition to what has been discussed, the present data reveal further evidence about the central nervous mechanisms involved in the adaptive processes of the male laboratory rat. A number of responses which are displayed initially in an unfamiliar situation are diminished during habituation while more self-directed and item-specific behaviors such as investigating are increased (2). These changes in the behavioral pattern might be due to an effect on GABA neurons, dopamine neurons or an interaction between these two systems in the various brain structures involved in the expression of exploratory behavior.

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