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Analysis of the Difference in the Behavioral Effects of Apomorphine in C57BL/6 and DBA/2 Mice

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VETULANI, J., M. SANSONE AND A. OLIVERIO. *Analysis of the difference in the behavioral effects of apomorphine in C57BL/6 and DBA/2 mice*. PHARMAC. BIOCHEM. BEHAV. 17(5) 967–971, 1982.—The influence of pimozide on the effects of apomorphine on locomotor activity and stereotypy was studied in two inbred strains of mice. In C57BL/6 mice, in which apomorphine did not produce stereotypy of gnawing, the biphasic effect of apomorphine on locomotor activity (hypomotility followed by hypermotility) was unaffected by pimozide. In DBA/2 mice, in which high doses of apomorphine produce hypomotility and compulsive gnawing, both these effects (but not hypomotility produced by low doses of apomorphine) were counteracted by pimozide. The results are consistent with the assumption that both strains of mice have separate inhibitory and stimulatory dopamine receptors mediating locomotor activity. In addition, DBA/2 but not C57BL/6 mice have dopamine receptors which mediate stereotypy and are sensitive to pimozide.

Apomorphine Locomotor activity Pimozide Dopamine receptors Stereotypy Interstrain differences

APOMORPHINE, a drug regarded as a standard agonist of dopamine receptors, has been widely used to investigate the dopaminergic mechanisms that are involved in the control of locomotion (cf. [5]). In the rat the effects of apomorphine are rather consistent and the dose-response relationship is triphasic: depression-stimulation-depression [10, 14, 15, 18, 21]. The multiphasic effect was attributed to the action of apomorphine on various types of dopamine receptors: inhibitory microgram doses are thought to stimulate presynaptic dopaminergic receptors [1, 9, 17], milligram doses—stimulatory extrastriatal dopamine receptors [2, 11, 20], and still higher doses—striatal dopamine receptors that mediate stereotyped gnawing [7]. This stereotypy appears to inhibit locomotion [10, 22, 23]. Studies on cerebral pharmacokinetics of apomorphine confirm the suggestion that the various effects of the drug are mediated by receptors localized in different brain areas [19].

The results in mouse are conflicting. Similarly as in rats microgram doses of apomorphine inhibit the locomotor activity, presumably acting on a presynaptic receptor [6], but also doses around 1 mg/kg, which act postsynaptically, produce hypomotility [8, 21, 24]. The still higher doses were reported to depress [16], not affect [12,21] or stimulate locomotion [3, 12, 27, 30]. The discrepancy in the results is most probably caused by differences in the reaction to apomorphine of mice of various strains [12].

It was reported in the preceding paper [24] that the dose-effect relationship for the action of apomorphine on locomotor activity is different in C57BL/6 and DBA/2 mice: in the former strain the action was biphasic, with lower doses inhibiting, and higher increasing or not affecting locomotion, while in DBA/2 mice apomorphine in the whole dose range (up to 8 mg/kg) dose-dependently inhibited the activity.

In this study we investigated the possible reason for this difference, testing the effect of a dopamine receptor blocking agent, pimozide, and assessing the apomorphine-induced gnawing behavior. Our results indicate that the inhibitory effect of high doses of apomorphine on the locomotor activity in DBA/2 mice is reversible by pimozide, and is concomitant with the appearance of stereotyped gnawing, a phenomenon absent in C57BL/6 mice.

METHOD

Animals

Male mice of inbred strains C57BL/6 and DBA/2, weighing 25–30 g, were purchased from Charles River, Calco Como. For at least one week before the experiment they were kept in animal house eight to a cage, under standard laboratory conditions (free access to food and water, ambient temperature of 22°C, 12–12 hr dark-light cycle). Only naive mice were used for testing locomotor activity, while in tests for stereotypy some mice previously investigated on locomotor activity (control groups) were employed.

Compounds

Apomorphine hydrochloride (Sandoz) dissolved in bidistilled water, and pimozide (Janssen), dissolved in 0.1% tartaric acid, were injected intraperitoneally in a volume of 10 ml/kg. The controls received appropriate solvents. Apomorphine was given 15 min before testing locomotor activity, or

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immediately before observing stereotyped behavior. Pimozide was given 90 min before the tests.

Locomotor Activity

The mice were tested in an apparatus consisting of 8 toggle-floor boxes, described in detail previously [24]. The number of crossings from one compartment to the other was recorded for 30 min. Each group consisted of eight mice.

The data were analyzed statistically by a two-factor analysis of variance. For analysis of pimozide-apomorphine interaction the analysis was carried out separately for each strain. The two factors were pimozide pretreatment (2 levels: 0 and 0.2 mg/kg) and apomorphine treatment (4 levels: 0, 1, 4 and 8 mg/kg). The analysis of the action of pimozide alone was carried out for both strains; the two factors were strain (2 levels) and pimozide dose (3 levels: 0, 0.3 and 0.5 mg/kg). Individual between group comparisons were carried out employing the error term of the overall analysis of variance [13].

Stereotyped Gnawing

Two types of observation permitting one to notice stereotyped gnawing were carried out:

(a) A mouse was placed in a 25×10×10 cm macrolone cage, without bedding, containing a slightly crumpled filter paper strip, approximately 25×1 cm. When the mouse attempted to bite at the filter paper, the whole strip moved, and the observer could easily notice if the movement was caused by biting or by accidental touching of the strip.

(b) A mouse was placed in a shallow aluminum disposable container 35×25×7 cm, covered with a translucent plate. A flexible rod made of pressed and rolled aluminum foil, tightly wrapped in a distended Parafilm® sheet was placed inside. Both the rod and the wall of the box were connected, without solding, to a drinkometer. Each closing of the circuit, which was possible only if the mouse bit through the film, was recorded. In this instrument some mice bit preferentially, if not exclusively, at the rod, while others tried to bite at the walls or the floor of the container. In the latter case the biting produced a characteristic sound that was easily perceived by the observer.

The presently used methods for detection of stereotypy could only yield results that were treated as all-or-none responses and evaluated with the Fisher exact probability test [28].

RESULTS

Locomotor Activity

Apomorphine produced in C57BL/6 mice a biphasic effect, apparently unchanged by pimozide pretreatment.

A two-factor analysis of variance has shown a significant apomorphine main effect, $F(3,56)=21.64$, $p<0.001$, while pimozide produced no significant effect, $F(1,56)=0.16$. No significant treatment × pretreatment interaction was found, $F(3,56)=1.74$, and therefore the significance of the difference between the control and apomorphine group was calculated disregarding the difference in pretreatment.

Locomotor activity was significantly depressed by the low dose of apomorphine (1 mg/kg), and significantly elevated by the high dose (8 mg/kg). It should be emphasized that the stimulatory action of the high dose of apomorphine appeared even more evident in pimozide than in solvent-pretreated mice (Fig. 1).

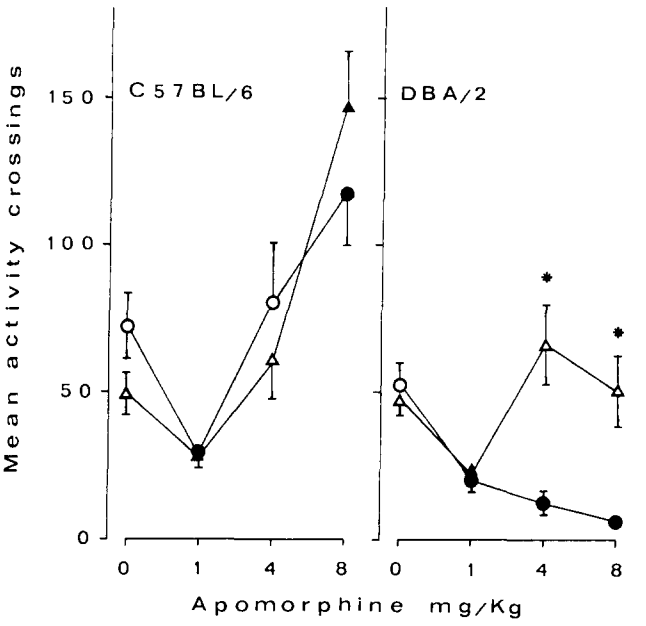

FIG. 1. Effects of apomorphine given alone (circles) or after pimozide (0.2 mg/kg) (triangles) on spontaneous locomotor activity (crossings) of C57BL/6 (left panel) or DBA/2 (right panel) mice during 30 min. Vertical bars indicate standard errors of the means. Full symbols denote a significant difference ($p<0.05$) vs saline (dose 0 of apomorphine). Asterisks denote a significant difference ($p<0.05$) vs the group receiving apomorphine without pimozide pretreatment.

In DBA/2 mice apomorphine depressed the locomotor activity monotonically, in a dose-dependent manner. Pimozide reversed the inhibitory effect of high doses, but not the hypomotility brought about by the low dose of apomorphine (Fig. 1).

A two-factor analysis of variance has shown significant main effects of pimozide and apomorphine, $F(1,56)=19.77$, $p<0.001$, and $F(3,56)=5.98$, $p<0.01$, respectively, and a significant pimozide × apomorphine interaction, $F(3,56)=7.68$, $p<0.001$.

Both strains of mice differed in their locomotor activity, $F(1,42)=7.4$, $p<0.01$, and the main effect of pimozide was highly significant, $F(2,42)=41.42$, $p<0.001$, but the strain × pimozide interaction was virtually nonexistent, $F(2,42)=0.13$. As seen from Table 1, pimozide in doses of 0.3 and 0.5 mg/kg reduced the locomotor activity in both strains of mice to a similar degree.

Stereotyped Behavior

General behavior. Apomorphine in doses of 4–10 mg/kg produced stereotyped behavior both in C57BL/6 and DBA/2 mice, but the type of behavior was different in these two strains. The stereotypy in C57BL/6 mice was characterized by no suppression of locomotor activity, but constant assumption of upright postures and intensive sniffing. Bites at the walls of the cage were very infrequent but strong. The stereotyped behavior in DBA/2 mice was characterized by suppression of locomotion and intensive but fine biting at the floor or the objects in the cage. The mice kept their heads low, so that the biting was difficult to observe if no special means were used.

TABLE 1

EFFECT OF PIMOZIDE ON LOCOMOTOR ACTIVITY IN C57BL/6 AND DBA/2 MICE

Pimozide dose (mg/kg)	Strain: C57BL/6 Mean ± S.E.M.	C57BL/6 % contr.	DBA/2 Mean ± S.E.M.	DBA/2 % contr.
0.0	65.50 ± 8.02	100	80.00 ± 10.93	100
0.3	19.50 ± 2.20	30	30.00 ± 3.98	37
0.5	13.75 ± 1.91	21	30.50 ± 5.22	38

Two-factor between-subjects ANOVA

Source	*df*	SS	MS	F	$p<$
Strain	1	2,324.083	2,324.083	7.406	0.01
Pimozide dose	2	25,993.500	12,996.750	41.415	0.001
Strain × Pimozide dose	2	80.167	40.083	0.128	
Error	42	13,180.283	313.816		
Total	47	41,578.033			

The data are mean numbers of crossings during 30 min. Each group consisted of 8 mice.

Filter paper strip biting. C57BL/6 mice receiving apomorphine in doses up to 24 mg/kg did not bite the strip during the 1 hr observation period. DBA/2 mice receiving 10 or 20 mg/kg of apomorphine invariably bit the strip constantly, leaving clearly detectable traces. Some of them bit the strip mainly in one place, leaving a hole in the paper, while others bit along the edges of the strip, producing a fine line of traces. In the group receiving 4 mg/kg apomorphine six out of eight mice bit the strip; of eight mice pretreated with 0.2 mg/kg pimozide no one bit the strip after treatment with 4 mg/kg apomorphine. This demonstrates that pimozide significantly counteracts the gnawing behavior ($p=0.0007$, Fisher test, two-tailed).

Aluminum foil rod biting. None of the five C57BL/6 mice receiving 16 mg/kg apomorphine bit the rod; occasionally the mice bit the wall of the container, making a clearly audible sound. There were no more than ten such bites during the 1 hr observation period.

DBA/2 mice receiving 4–16 mg/kg apomorphine bit either the box floor or the rod. A preliminary study, in which the number of bites at the rod was recorded electronically, demonstrated that the mice receiving 16 mg/kg apomorphine may be divided into three groups in respect to the frequency of response: intensive biters (570–1401 bites/hr, mean 870.7, 6 out of 16), moderate biters (73–132 bites/hr, mean 120.0, 5 out of 16), and low biters (7–50 bites/hr, mean 22.2, 5 out of 16). An experiment repeated with the same dose of apomorphine 48 hr later has revealed that the intensity of biting at the rod seems to be a stable characteristic: all intensive biters were performing high (727–2074 bites/hr, mean 1161.8), while of the 5 low biters four remained at the low level (0–42 bites/hr), and only one became a moderate biter (306 bites/hr). It should be emphasized that the mice which did not bite at the rod, bit intensively at the floor of the container, making a clearly audible scratching sound.

DISCUSSION

Our present results indicate that apomorphine produces stereotypy of biting in some, but not all strains of mice, and suggest that this response may influence the effect of high doses of apomorphine on locomotor activity. In the strains in which apomorphine produces stereotypy of biting, it dose-dependently inhibits the locomotor activity, while in the strains not responding with this type of stereotypy the effect of apomorphine on locomotor activity is biphasic.

The stereotypy of biting in mice is very inconspicuous and may elude visual observation. Thus, Lapin [12] reported that apomorphine produced no stereotypy of biting in five strains of mice, and in our preceding study [24] we were unable to notice stereotyped gnawing in DBA/2 mice when observations were carried out through the top of a shuttlebox. However, even when a method visualizing bites is employed, the responses of mice vary. Thus, Scheel-Krüger [25] has observed that apomorphine given alone produces moderate biting in N.M.R.I. mice, but others, using Kausali mice [4] or mice of an undefined strain [29], have found that apomorphine alone did not evoke biting response.

Apparently, the development of stereotypy of biting may explain the inhibitory effect of high doses of apomorphine on locomotor activity. Thus, these doses did not produce hypomotility in C57BL/6 mice, not responding with the stereotypy of biting, while depressed the locomotor activity of DBA/2 mice, in which the stereotypy of biting developed. Moreover, pimozide given in a dose that did not affect the locomotor activity of saline-treated mice of either strain but counteracted the stereotypy of biting in DBA/2 mice, antagonized also in this strain the hypomotility induced by high doses of apomorphine and change the dose-response pattern, rendering it similar to that characteristic of C57BL/6 mice. In

the latter strain pimozide did not alter the apomorphine dose-response curve.

It should be noted that pimozide acted differently in DBA/2 and C57BL/6 mice only in respect of interaction with high doses of apomorphine, while similarly in both strains did not affect the action of low doses of the drug and inhibited the spontaneous locomotor activity.

Gianutsos and Moore [8] have suggested that the effect of dopaminomimetics on the locomotor activity of Swiss-Webster mice is mediated by two types of postsynaptic receptors: inhibitory, interacting with all dopaminomimetics, and stimulatory, activated under normal conditions only by higher doses of some dopaminomimetics, apomorphine including. Our results suggest that a similar situation may exist in the case of C57BL/6 mice. As pimozide did not affect either hypo- or hypermotility phase, both the receptors seem to be relatively unresponsive to neuroleptics. In DBA/2 mice the action of apomorphine on locomotor activity may be additionally modified by a third dopaminergic receptor, mediating stereotypy of biting. The stimulation of this receptor produces inhibition of locomotor activity that masks the effect of interaction of apomorphine with the stimulatory receptor. Apparently both these receptors are similarly sensitive to apomorphine (less sensitive than the inhibitory receptor), but they differ in responsiveness to pimozide. Similarly as in rats, in which neuroleptics preferentially block stereotypy of gnawing over locomotor effects of apomorphine [14], in DBA/2 mice pimozide preferentially blocks the receptor mediating stereotypy, thus unmasking the stimulatory effect of apomorphine.

By analogy with rats [7] it might be supposed that in mice also the receptor responsible for stereotyped gnawing is present in the striatum. If this is correct, striatal dopaminergic receptors in various strains of mice may be different. In fact, Severson *et al.* [26] have reported relatively low concentration of ^{3}H/spiroperidol binding sites in the striatum of C57BL/6 mice.

To conclude, we suggest that three types of postsynaptic dopamine receptors may control the effect of apomorphine on locomotor activity of mice. They are: (1) an inhibitory receptor of unknown localization, sensitive to low concentrations of various dopaminomimetics, relatively insensitive to pimozide; (2) a stimulatory receptor, sensitive only to some dopaminomimetics, less sensitive to apomorphine than the inhibitory receptor, relatively insensitive to neuroleptics, possibly analogous to the stimulatory extrastriatal dopamine receptors in the rat [2,20]; and (3) striatal, stereotypy mediating receptor, relatively insensitive to apomorphine but very sensitive to pimozide. This third receptor is present only in some strains of mice and antagonizes the effect of stimulation of the stimulatory receptor. In mice strains having this receptor apomorphine produces monotonous, dose-dependent inhibition of locomotor activity, while in the strains lacking this receptor apomorphine evokes a biphasic effect on locomotion.

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