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Dopamine Receptors in the Striatum and Limbic System of Various Strains of Mice: Relation to Differences in Responses to Apomorphine

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MICHALUK, J., L. ANTKIEWICZ-MICHALUK, A. ROKOSZ-PELC, M. SANSONE, A. OLIVERIO AND J. VETULANI. *Dopamine receptors in the striatum and limbic system of various strains of mice: Relation to differences in responses to apomorphine.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1115–1118, 1982.—Dopamine receptors, defined as [^{3}H]spiroperidol binding sites, had similar population parameters in the limbic forebrain of C57BL/6, Albino Swiss and DBA/2 mice, but the parameters of the striatal populations were different: not only the densities differed among themselves, but the K_D value of the striatal dopamine receptors of DBA/2 mice was significantly higher than that in the two remaining strains. Behavioral responses of Albino Swiss mice to apomorphine: biphasic effect of apomorphine on locomotor activity and stereotypy characterized by high motility, frequent rearing and sharp, not very frequent bites, were similar to those described earlier for C57BL/6 mice, and differed from those reported for DBA/2 mice. The results suggest that the difference in responding to apomorphine in various strains of mice may be related to differences in their striatal dopamine receptors.

Striatal and limbic dopamine receptors Strain differences Locomotor activity Apomorphine stereotypy

APOMORPHINE, a standard agonist of dopamine receptors (cf. [6]), acts differently in various strains of mice. We have reported previously [19] that it produces stereotypy of biting in DBA/2 mice, while in C57BL/6 mice the apomorphine-induced stereotyped behavior is characterized by increased rearing, but virtually no biting was observed. The drug also has different affects on locomotor activity, inhibiting it monotonously in DBA/2 mice, while in C57BL/6 mice a biphasic response was observed: the lower doses depressed, but higher ones elevated locomotion [17]. We have hypothesized that the differences in responses to apomorphine may be caused by differences in characteristics of central dopamine receptors in these strains of mice. To test this we presently characterized the populations of [^{3}H]spiroperidol binding sites in the striatum and limbic forebrain of these two strains. In addition, we investigated the [^{3}H]spiroperidol binding sites in the brain of another mouse strain, Albino-Swiss, and tested the responses of mice of this strain to apomorphine.

The results suggest that the three strains have similar dopamine receptors in the limbic forebrain, but display different parameters in the striatum, and that these differences may be related to their behavioral response to apomorphine.

METHOD

Inbred male DBA/2 and C57BL/6 mice (23–28 g) were purchased from Charles River (Calco Como, Italy). Random bred male Albino-Swiss mice (25–40 g) were obtained from licensed dealers in Poland.

Behavioral Study

Locomotor activity of Albino-Swiss mice was measured using circular actometers, 24 cm in diameter and 12 cm high, with two crossing light beams illuminating photoresistors. They were similar in construction to rat actometers described earlier [1] but had no central pole. The mice were placed in actometers individually, 15 min after injection of various doses of apomorphine (hydrochloride; Sandoz) and the number of beam crossings was recorded every 10 min for 30 min. The controls received saline, 10 ml/kg IP.

The data were used to assess the total activity (counts between 0 and 30th min), exploratory activity (0–10th min), and basal locomotor activity (10–30th min).

Stereotyped behavior was observed as previously described [19], by placing a mouse in a plastic box without bedding, containing a pleated filter paper strip; biting at the

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strip was easily observable. The mice were put into boxes immediately after apomorphine injections and were kept there for 60 min.

Receptor Assay

The mice were decapitated and the brains were rapidly excised and placed on an ice-cooled porcelain plate. The striatum and the limbic forebrain were excised, using a procedure similar as that described earlier in the rat [2]. The brain part described as "the limbic forebrain" contained olfactory tubercles, rostral limbic nuclei, nucleus accumbens and other septal nuclei. The tissue was stored under solid CO_2 until used.

Membranes were prepared at 0–4°C. The tissue was homogenized in a Polytron homogenizer (setting 4, 10 sec) in 20 vol. of 50 mM Tris-HCl buffer pH 7.6, containing 0.1% ascorbic acid. The homogenate was centrifuged at 1,000 g for 10 min, the pellet was discarded, and the supernatant was centrifuged at 43,500 g for 30 min. The pellet (P_2) was reconstituted in the original volume of Tris-HCl buffer and recentrifuged under the same conditions.

The pellets were stored frozen (−80°C) till assayed. For the assay the pellets from the striatum were suspended in the Tris-HCl buffer; the final membrane preparation contained 15–20 mg protein (assayed according to Lowry *et al.* [12] per milliliter. The pellets from the limbic forebrain were suspended in 50 mM Tris-HCl buffer pH 7.6 containing 0.1% ascorbic acid and 10 μM pargyline (to prevent serotonin oxidative desamination); the final preparation contained 28–30 mg protein per milliliter.

The incubation mixture for striatal membranes contained 500 μl of membrane suspension, 100 μl of [^{3}H]spiroperidol (5 or 6 concentrations, 0.07–4 nM), 200 μl of Tris-HCl buffer containing ascorbic acid, and 200 μl of 10 μM spiroperidol in the buffer (blanks) or the buffer (total binding samples). The total volume of the sample was 1.00 ml. The incubation mixture for limbic forebrain membranes was similar, but Tris-HCl buffer contained additionally 10 μM pargyline, and the samples contained 100 μl of 10 μM serotonin in the buffer to prevent [^{3}H]spiroperidol binding to serotonin binding sites. The total volume was also 1.00 ml.

The incubation was carried out at 37°C for 10 min and was terminated by cooling for 10 min in ice-cold water and filtration through Whatman GF/C filters under reduced pressure. The tubes were washed twice with 5 ml portions of ice-cold Tris-HCl buffer and placed in scintillation vials, in 8 ml of Bray's scintillator. The radioactivity was measured in a Packard model 3255B liquid scintillation counter, at 42% yield.

The data were analyzed by Scatchard analysis. The statistical significance of results was assessed with the one way analysis of variance followed, if appropriate, by Dunnett's test [8].

RESULTS

Locomotor Activity of Albino Swiss Mice

Explorative activity of Albino Swiss mice was approximately twice as high as their basal activity (Fig. 1). Apomorphine in the investigated dose range significantly affected both types of activity, particularly the latter. The dose-effect relationship was biphasic: low doses depressed the locomotor activities, while high ones facilitated them. The differences in basal but not exploratory activity were significant when compared with controls; there was no close parralellism between the maximal effects of apomorphine on explorative and basal activities.

The changes in basal activity determined the shape of the dose-response relationship for the total locomotor activity, although the effects of apomorphine on the latter were less pronounced.

Stereotyped Behavior of Albino Swiss Mice

Mice receiving 16 mg/kg apomorphine displayed distinct stereotyped behavior, commencing approx. 5 min after the injection. The animals were aroused, hyperreactive, showed frequent rearing and occasionally jumped. They sniffed the filter strip and bit it in a characteristic manner, as if attacking the strip, but never displayed continuous chewing. In spite of apparent aggressiveness, when paired the mice did not fight, but rather did not appear to notice the partner.

[^{3}H]Spiroperidol Binding Sites in the Striatum and Limbic System of Albino Swiss, C57BL/6 and DBA/ Mice

As shown by correlation coefficient values, the Scatchard plots of [^{3}H]spiroperidol binding were always rectilinear, indicating a single population of binding sites (Table 1). The differences in parameters of populations in the limbic forebrain (B_{max} and K_D) in various strains did not reach the level of statistical significance. On the other hand, differences were observed in striatal [^{3}H]spiroperidol binding sites: all three strains differed among themselves significantly in respect to the density of the sites, and the affinity of these sites in DBA/2 mice was significantly lower than the affinities of the binding sites in Albino Swiss and C57BL/6 mice.

The three investigated strains differed also in respect to the relation between their striatal and limbic [^{3}H]spiroperidol binding sites. In Albino Swiss mice the density of these sites in both brain regions was virtually the same, but the affinity of the striatal sites was over 5 times higher than that in the limbic forebrain. Similarly, in C57BL/6 mice the affinity of [^{3}H]spiroperidol binding sites in the striatum was approximately 3.5 times higher than that in the limbic forebrain, and moreover the density of the striatal sites was twice as high. In contrast, in DBA/2 mice the affinity of the striatal sites, which was lower by 3–10 times than in the other strains, was lower than the affinity of the limbic sites.

DISCUSSION

Different strains of mice react dissimilarly to various agonist of central receptors for neurotransmitters, such as morphine [4,14], clonidine [9], or 3-chlorophenylpiperazine [18], stimulation of opiate, α_2-adrenergic or serotonin receptors, respectively. Our previous reports indicate that C57BL/6 and DBA/2 mice react differently to a dopamine receptor agonist, apomorphine [17,19]. These results suggest that mice of various strains may differ in respect to the characteristics of populations of various cerebral receptors.

The present study indicates that this is true for dopaminergic receptors in the striatum: the K_D values for the striatal [^{3}H]spiroperidol binding sites (that in our experimental conditions correspond with dopamine receptors [3,10]) in DBA/2 mice were significantly different from those in C57BL/6 and Albino-Swiss mice. In addition, there were also significant differences among all strains in the density of striatal dopamine receptors.

The mesolimbic dopamine receptors in these strains differed significantly (in respect to their K_D) from striatal recep-

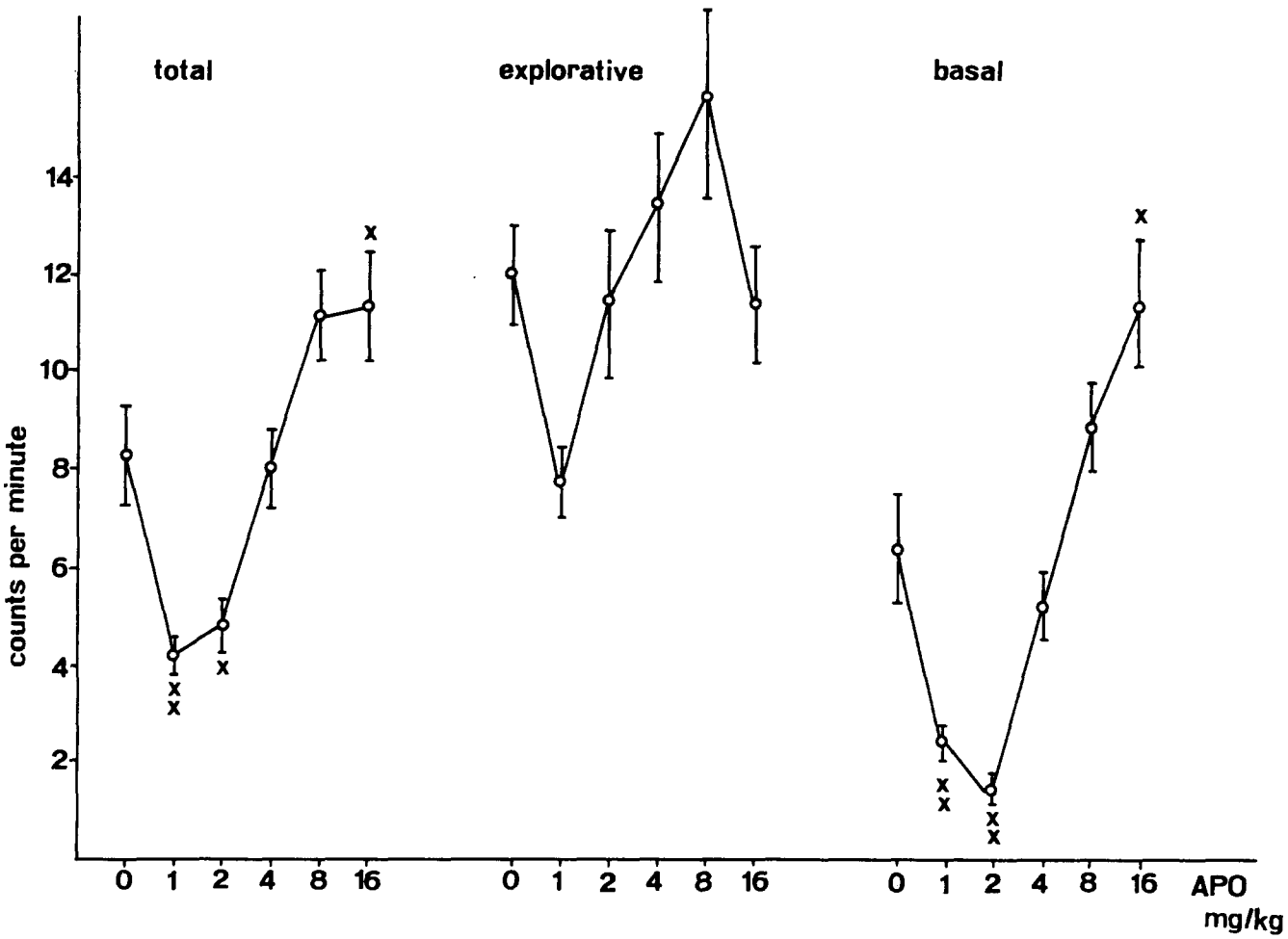

FIG. 1. Effect of various doses of apomorphine (APO) on total, explorative and basal locomotor activity of Albino Swiss mice. Each point represents the mean of 7–9 measurements. Vertical bars denote S.E.M. The effect of apomorphine was significant for each kind of locomotor activity: the values of F were 9.09 for total, 5.09 for explorative, and 13.33 for basal activity (*df* 5,45). $^{x}p<0.05$, $^{xx}p<0.01$ (difference from controls, Newman-Keuls test).

TABLE 1

CHARACTERISTICS OF [^{3}H]SPIROPERIDOL BINDING SITES IN THE STRIATUM AND LIMBIC FOREBRAIN OF VARIOUS STRAINS OF MICE

	Striatum				Limbic forebrain			
Strain	N	B_{max} (fmol/mg prot)	K_D (nM)	r^2	N	B_{max} (fmol/mg prot)	K_D (nM)	r^2
Albino-Swiss	5	108.4 ± 10.8*	0.33 ± 0.07	0.88	5	106.8 ± 14.8	1.88 ± 0.35‡	0.90
C57BL/6	10	399.4 ± 12.0*	0.96 ± 0.05	0.98	9	183.6 ± 22.0†	3.43 ± 0.49†	0.88
DBA/2	11	248.7 ± 17.0*	3.26 ± 0.28*	0.94	5	147.2 ± 34.1‡	2.04 ± 0.59§	0.80
		F(2,23)=73.12	F(2,23)=54.57			F(2,16)=2.46	F(2,16)=3.11	

The data are means±S.E.M.
*Significantly different from the remaining strains, $p<0.01$ (Newman-Keuls test).
†Significantly different from the striatal value, $p<0.001$, ‡$p<0.01$, §$p<0.05$ (F test).

tors, but no significant inter-strain differences were observed.

As the responses of C57BL/6 and DBA/2 mice to apomorphine were reported previously to be different [17,19], it was of interest to find out if Albino Swiss mice react to apomorphine similarly to either of the inbred strains. The present results indicated that the response of Albino Swiss mice to apomorphine follows the pattern observed in C57BL/6 mice: biphasic dose-response relationship for locomotor activity and stereotypy with predominant motor stimulation and the absence of fine gnawing at the filter paper strip. It might be noted that the biphasic effect of apomorphine on locomotor activity is particularly expressed for basal locomotor activity, while much less evident for exploratory activity. This seems to support the notion that the effect is mediated through dopaminergic receptors, which seem to be involved in the control of basal locomotor behavior, while noradrenergic system is involved mostly in motivated behavior, including exploration (cf. [1]).

The similarity in behavioral response to apomorphine between Albino Swiss and C57BL/6 mice is paralleled by the similarity in the characteristics of striatal dopamine receptors. Although the densities of these receptors differ significantly, the difference in the K_D did not reach the level of statistical significance. It seems convincible, therefore, that the differences in the behavioral responses to apomorphine between these strains of mice on one hand, and DBA mice on the other are related to the differences in striatal receptors.

Pharmacological [7], and pharmacokinetic [13] studies indicated that the striatal dopamine receptors in the rat are predominantly involved in the apomorphine-induced stereotypy of biting, while locomotor effects of apomorphine were associated with the dopaminergic mesolimbic system [5,15]. It is well known, however, that the appearance of stereotypic gnawing is accompanied by a decrease in locomotion [11,16], and therefore it seems that the striatal dopamine system may modulate the effects of stimulation of the dopamine mesolimbic receptors. This view seems to be corroborated by the present findings that differences in the pattern of motor responses to apomorphine exist between the strains having different striatal, but similar mesolimbic receptor populations.

The fact that various strains of mice differ among themselves with regard to central receptors for neurotransmitters as well as of behavior and drug responsiveness suggests that the inbred strains of mice may be particularly useful for studying the relationships between neurotransmitters, receptor systems and behavior.

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