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Potency and Efficacy of Dopamine Agonists in Mouse Strains Differing in Dopamine Cell and Receptor Number

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SHANNON, H. E., K. G. BEMIS AND S. C. PETERS. *Potency and efficacy of dopamine agonists in mouse strains differing in dopamine cell and receptor number.* PHARMACOL BIOCHEM BEHAV **40**(1) 103–107, 1990.—The potency and efficacy of the selective dopamine D2 receptor agonist quinpirole, the mixed D1/D2 agonist apomorphine, and the selective D1 receptor agonist SKF 38393 in producing hypothermia and changes in locomotor activity were evaluated in four strains of mice: CBA/J, C57BL/6J, ICR Swiss and CF1. CBA/J mice previously have been shown to be deficient in dopamine cell and receptor number relative to other strains such as C57BL/6J mice, whereas ICR Swiss and CF1 are commonly used strains of mice. Quinpirole (0.125 to 1.0 mg/kg) was equiefficacious and equipotent in producing hypothermia in all 4 strains. Apomorphine (0.125 to 16 mg/kg) was equiefficacious in producing hypothermia in all 4 strains, but was approximately four-fold less potent in CBA/J mice than in the other strains. SKF 38393 had little effect on body temperature in any of the 4 strains. Basal motor activity was lowest in CBA/J mice, and tended to be highest in ICR Swiss mice. Quinpirole (0.125 to 32 mg/kg) had no effect on motor activity in CBA/J mice, but decreased motor activity in the other 3 strains. Apomorphine (1 to 16 mg/kg) produced modest increases in motor activity in all 4 strains. The magnitude of the changes produced by apomorphine was comparable in all strains when expressed as change from mean control values. SKF 38393 (8 to 64 mg/kg) also increased motor activity in all 4 strains, with comparable increases when expressed as change from mean control values. The present results are consistent with the interpretation that inherited deficiencies in dopamine cell and receptor number in CBA/J mice produce functional decrements in D2, but not D1, dopamine receptor function.

D1 receptor D2 receptor Mouse Motor activity

THE number of dopaminergic neurons, as well as the activity of the catecholamine synthesizing enzyme tyrosine hydroxylase, are substantially less in the midbrain of CBA/J mice than in other strains such as C57BL/6J and BALB/cJ mice (2, 6, 14). Further, the concentration of dopamine receptors in the striatum of CBA/J mice is less than in BALB/cJ or C57BL/6J mice (4,21). Moreover, prolactin levels are higher in CBA/J than in BALB/cJ mice (3). Concordantly, the pharmacologic effects of dopaminergic drugs differ in CBA/J mice as compared with other strains. Altered responsiveness to amphetamine and apomorphine in producing stereotyped behavior and hyperactivity have been reported in CBA/J mice compared with C57BL/6J or BALB/cJ mice [(6,16); but see (13)]. Neuroleptics are more effective in producing catalepsy in CBA/J mice than in C57BL/6J or BALB/cJ mice (7,16). Further, chronic treatment with haloperidol produced supersensitivity to apomorphine in C57BL/6J and BALB/cJ mice but not CBA/J mice (16). However, only nonselective dopamine agonists have been evaluated in CBA/J mice. The effects of selective D2 and D1 agonists in CBA/J mice, as compared to other strains, are unknown.

The purpose of the present study was to compare the efficacy and potency of the selective D2 receptor agonist quinpirole, the selective D1 receptor agonist SKF 38393 and the mixed D1/D2 agonist apomorphine in producing changes in body temperature and motor activity in CBA/J, C57BL/6J, and the more commonly used ICR Swiss, and CF1 (a non-Swiss strain) mice.

METHOD

Subjects

Male CBA/J, C57BL/6J (Jackson Laboratories, Bar Harbor, ME), ICR Swiss (Harlan Sprague-Dawley, Indianapolis, IN) and Crl:CF1®BR (Charles River Laboratories, Portage, MI) mice weighing 20–30 grams were housed in groups of 12 in a large colony room with food and water available continuously. The lights in the colony room were turned on between 6:00 a.m. and 6:00 p.m. Studies were conducted between 8:00 a.m. and 4:00 p.m. in a quiet room.

Body Temperature

Rectal temperature was measured (Model BAT 8, Bailey Instruments, Saddle Brook, NJ) immediately before and 30 min

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after an IP injection of vehicle, or a dose of quinpirole, SKF 38393 or apomorphine. Five mice were used per group.

Locomotor Activity

Locomotor activity was measured with 8 Digiscan Animal Activity Monitors (Model RXYZ, Omnitech Electronics, Inc., Columbus, OH). Interruptions of any of the infrared photocells were recorded by an Apple II+ computer. Mice were placed individually in a polypropylene cage (28.5 × 17.5 × 12 cm high), and habituated to this cage for 30 min before an IP injection of vehicle, or a dose of quinpirole, SKF 38393 or apomorphine. Motor activity was then recorded for 60 min. Eight mice were used per group, although data for some mice were lost due to equipment malfunction.

Data Analysis

Changes in rectal body temperature (°C) were calculated by subtracting the baseline temperature from the temperature obtained 30 min after vehicle or drug administration. Locomotor activity was recorded as the number of inches traversed during the 60-min observation period. A square-root transformation was applied before statistical analysis to obtain homogeneity of variance. Changes in locomotor activity were calculated by subtracting the mean of the respective control group from the value obtained for each mouse.

The dependent variables temperature change and inches traversed were both analyzed by a two-way analysis of variance. The two factors were drug dose and strain of mice. An interaction term also was included to evaluate if the dose-response curves differed among the mouse strains. Because drug dose is a quantitative factor it was partitioned into independent (orthogonal) components (9). These orthogonal components were as follows: 1) drug doses versus vehicle, which is the mean response over all doses versus the mean response for vehicle, and is referred to as the main effect for dose, 2) linear, 3) quadratic, and 4) quartic (if there were sufficient degrees of freedom) trend analysis components. The dose × strain interaction term also was partitioned to evaluate the differences among the mouse strains. The independent components and their interactions were the effects used to report significance. In some experiments, dose groups were compared to their respective vehicle-treated group using the Least Significant Difference test based on analysis of variance. A p-value of <0.05 was taken as the level of statistical significance. All computations were done with the SAS System using PROC GLM (SAS User's Guide: Statistics, 1985).

Drugs

The following drugs were used in this study: quinpirole HCl (Eli Lilly and Co., Indianapolis, IN), apomorphine HCl (Sigma Chemical Co., St. Louis, MO) and +SKF 38393 (+-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol) HCl (Research Biochemicals, Natick, MA). Drug doses are expressed as mg/kg of the drug form listed. The vehicle for all drugs was distilled water. All injections were given IP in a volume of 10 ml/kg.

RESULTS

Body Temperature

The selective D2 agonist quinpirole (0.125 to 1.0 mg/kg) produced dose-related decreases in body temperature in all four strains of mice (Fig. 1, upper panel). Quinpirole was approximately equiefficacious and equipotent in the four strains of mice in producing hypothermia. In the analysis of variance, the main effect for dose was significant, $F(1,80)=87$, $p=0.001$, and decreases in temperature were significantly related linearly to log-dose, $F(1,80)=244$, $p=0.0001$. There were no statistically significant differences in the effects of quinpirole among the four strains.

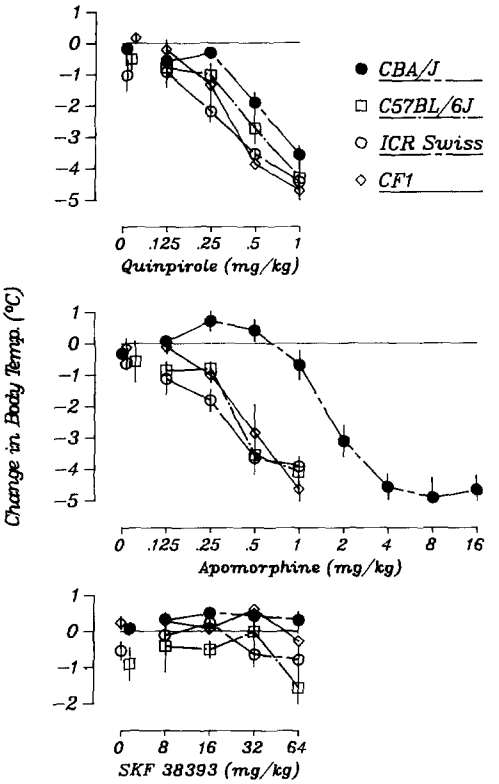

FIG. 1. Dose-dependent changes in body temperature produced by quinpirole, apomorphine and SKF 38393 in four strains of mice. Changes in body temperature following the administration of vehicle alone are represented by the points above 0 mg/kg. Each point represents the mean of 5 mice. The vertical lines represent ±1 S.E.M.

The mixed D1/D2 agonist apomorphine (0.125 to 16 mg/kg) also produced a dose-related decrease in body temperature (Fig. 2, middle panel). There were no substantial differences in maximal decreases in body temperature produced by apomorphine in the four strains of mice. However, apomorphine was approximately four-fold less potent in decreasing body temperature in the CBA strain as compared with the other strains. In the analysis of variance, the main effect for dose was significant, $F(1,80)=63$, $p=0.0001$, and decreases in temperature were significantly related linearly to log dose, $F(1,80)=128$, $p=0.0001$.

In contrast, the selective D1 agonist SKF 38393 did not produce substantial changes in body temperature. In the analysis of variance, the main effect for dose was not statistically significant, $F(1,80)=0.99$, $p=0.32$.

Locomotor Activity

Over the dose-range of 0.125 to 1.0 mg/kg (Fig. 2, upper left panel), quinpirole produced dose-related decreases in locomotor activity in the C57, ICR and CF1 strains, but was with-

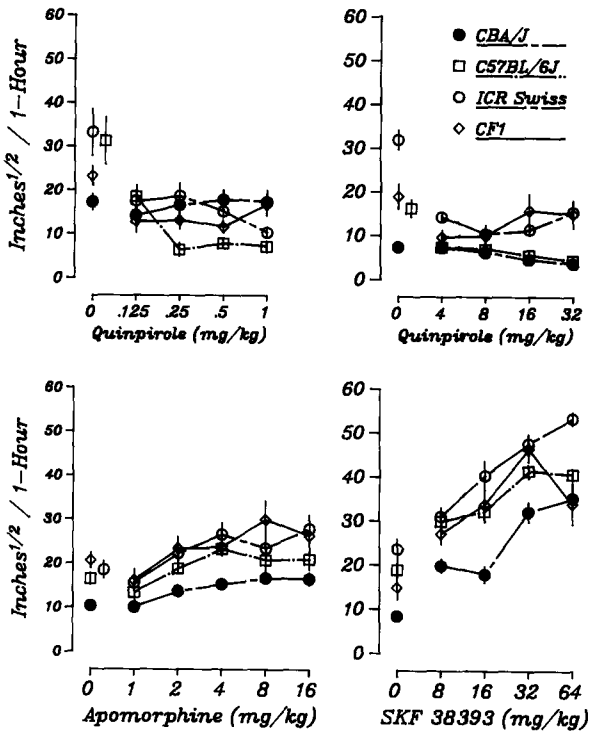

FIG. 2. Dose-response curves for quinpirole, apomorphine and SKF 38393 on locomotor activity in four strains of mice. Activity following the administration of vehicle alone are represented by the points above 0 mg/kg. Each point represents the mean of one observation in each of six to eight different mice. The vertical lines represent ±1 S.E.M.

out effect in the CBA strain. In the analysis of variance, there was a significant difference in the dose-related decreases among the 4 strains [linear × strain: $F(3,131)=4.0$, $p=0.0096$]. In addition, the effects of quinpirole also were examined over the dose-range of 4 to 32 mg/kg of quinpirole. These higher doses of quinpirole reduced activity in all 4 strains, but the decreases were significant only in the C57, ICR, and CF1 strains (Least Significant Difference test, comparing each dose in each strain to the respective vehicle control group).

Apomorphine (1 to 16 mg/kg) produced dose-related increases in locomotor activity in all four strains (Fig. 2, lower left panel). The absolute values of the increases were greatest in the CF1 strain, and smallest in the CBA strain. However, when the data were expressed as changes from the mean of the respective control groups, there were no substantial differences among the four strains (Fig. 3, left panel), indicating that relative to basal activity levels, apomorphine was approximately equieffective and equipotent in all four strains. In the analysis of variance, the main effect for dose was significant, $F(1,166)=8.8$, $p=0.0034$, and changes in locomotor activity were significantly related linearly to log-dose, $F(1,166)=37$, $p=0.0001$.

Like apomorphine, SKF 38393 produced dose-related increases in locomotor activity. Doses of 8 to 32 mg/kg increased activity in all four strains (Fig. 2, lower right panel). A dose of 64 mg/kg produced further increases in the CBA and ICR strains, but increased activity less in the CF1 and C57 strains. When the data were expressed as changes from the mean of the control group (Fig. 3, right panel), the largest changes occurred in the CF1 strain, but there were no significant differences

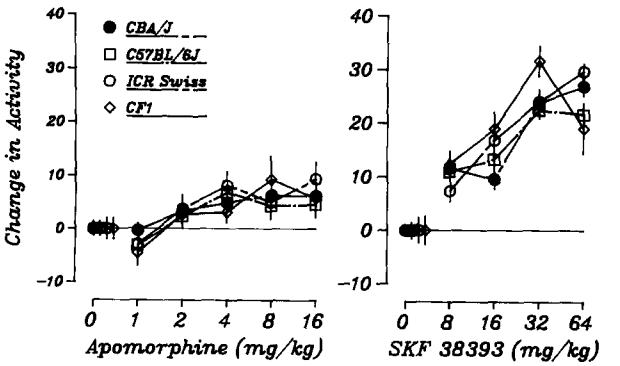

FIG. 3. Dose-dependent effects of apomorphine and SKF 38393 on the changes in locomotor activity relative to the mean of the respective control groups in four strains of mice. Activity following the administration of vehicle alone are represented by the points above 0 mg/kg. Each point represents the mean of one observation in each of six to eight different mice. The vertical lines represent ±1 S.E.M.

among the strains. In the analysis of variance, the main effect due to dose was significant, $F(1,140)=193$, $p=0.0001$, and changes in locomotor activity were significantly related linearly to log-dose, $F(1,140)=93$, $p=0.0001$.

DISCUSSION

CBA/J mice have fewer dopamine cells and receptors than do other strains such as C57BL/6J mice (2, 6, 14). The functional consequences of this inherited deficiency include higher basal prolactin levels (3,22), altered sensitivity to the behavioral effects of amphetamine and apomorphine [(6,16); but see (13)], and increased sensitivity to the cataleptic effects of neuroleptics (7,16). In the present studies, CBA/J mice were less sensitive to both the hypothermic effects of the mixed D1/D2 agonist apomorphine, and to the activity-decreasing effects of the D2 agonist quinpirole. On the other hand, CBA/J mice were not differentially sensitive to the hypothermic effects of quinpirole, nor to the activity-increasing effects of the D1 agonist SKF 38393.

The selective D2 dopamine agonist quinpirole, and the mixed D1/D2 agonist apomorphine produced hypothermia in all four strains of mice. The selective D1 agonist SKF 38393 was without effect on body temperature in any of the four strains. The present results corroborate and extend previous studies demonstrating that the hypothermic effects of dopamine agonists are mediated by D2 receptors (11,15). In the present studies, the magnitude of the hypothermia produced by quinpirole and apomorphine was comparable in all four strains, demonstrating that although the number of dopamine receptors is less in CBA/J mice, the size of the receptor population in thermoregulatory centers is sufficient to mediate a full effect. On the other hand, although quinpirole was approximately equipotent in all 4 strains, apomorphine was approximately 4-fold less potent in CBA/J mice than in the other three strains. The mixed D1/D2 pharmacology of apomorphine may explain this difference in potency. A substantial body of literature now exists demonstrating that D1 and D2 receptors interact functionally. For example, stimulation of D1 receptors with SKF 38393 attenuates, whereas antagonism of D1 receptors with SCH 23390 enhances, the hypothermic effects of quinpirole (11,15). Since the decrease in receptor number in CBA/J mice has been defined by a decrease in B_{max} for ^{3}H-spiroperidol and ^{3}H-ADTN, ligands which would be ex-

pected to preferentially label D2 receptors, it is likely that, in CBA/J mice, there is a relatively selective loss of D2 receptors, leaving a relative excess of D1 receptors. Taken together, these results are consistent with the interpretation that the lower potency of apomorphine was due to a relatively greater physiological opposition of D2 receptor function by D1 receptor function.

Quinpirole decreased locomotor activity in the C57, ICR and CF1 strains, but failed to significantly decrease activity in the CBA/J mice. Since the baseline levels of activity in the CBA mice were lower than in the other 3 strains, quinpirole may have failed to decrease activity in the CBA strain due to a floor effect. On the other hand, in the experiment with the lower doses of quinpirole (0.25–1.0 mg/kg), activity levels in the C57 strain were reduced by quinpirole to values approximately half of those obtained with the CBA strain, indicating that it was possible to decrease activity further. Alternatively, then, these locomotor activity data suggest that CBA/J mice are deficient in D2 dopamine receptor function in motor areas of brain. This latter conclusion is consistent with the findings of Fink and Reis (6) that the number of dopamine cell bodies as well as the activity of tyrosine hydroxylase in nigrostriatal and mesolimbic dopaminergic systems are substantially decreased in CBA/J mice.

The mixed D1/D2 agonist apomorphine increased locomotor activity in all four strains. The absolute increases in activity were smallest in the CBA/J mice, but these mice also had the lowest basal activity. When data were expressed as changes from the mean of the control group, apomorphine was equipotent and equieffective in all four strains. Severson et al. (16) reported that a dose of 1.0 mg/kg of apomorphine produced lower stereotypy scores in CBA/J mice than in C57BL/6J or BALB/cJ mice. In contrast, Fink and Reis (6) reported that, over a range of doses, apomorphine produced higher stereotypy scores in CBA/J than in BALB/cJ mice. Further, Randall and Randall (13) demonstrated that sensitivity to apomorphine in CBA/J, C57BL/6J and BALB/cJ mice is dependent upon the subcomponents of the stereotyped behavior being measured. Taken together, these results suggest that there are few quantitative differences in the effects of apomorphine on motor behavior among the above referenced strains of mice.

In the present studies, the selective D1 agonist SKF 38393 produced dose-dependent increases in locomotor activity in all four strains of mice. The present results differ from previous reports that SKF 38393 has little effect on locomotor activity in normal animals (19), although it markedly increases activity in animals with supersensitive dopamine receptors (1,18). However, in animals habituated to the test environment, Molloy and Waddington (12) demonstrated that SKF 38393 increased locomotor, sniffing, rearing and grooming responses in rats using a behavioral check list technique. Starr and Starr (17) corroborated these latter findings, demonstrating that SKF 38393 increased grooming in mice habituated to the test environment. The present studies extend these previous reports by demonstrating that in mice habituated to the test environment, SKF 38393 increases locomotor activity. It would be of interest to determine whether SKF 38393 also increases locomotor activity in rats habituated to the test environment. The magnitude of the SKF 38393-induced increases in locomotor activity was not substantially different among the four strains when the data were expressed as changes from the means of the respective control groups. SKF 38393 was approximately equipotent in all 4 strains of mice. These results are consistent with the interpretation that CBA/J mice are not deficient in D1 receptor function relative to other mouse strains. Further data regarding specific D1 and D2 receptor binding and receptor-effector coupling in CBA/J mice in comparison to other strains are needed to further substantiate this hypothesis.

In summary, the dopamine agonists quinpirole and apomorphine produced comparable magnitudes of hypothermia, an effect mediated by D2 receptors, in CBA/J mice, a strain which is deficient in dopamine cells and receptors, and in three other strains of mice. However, apomorphine was less potent in producing hypothermia in CBA/J mice then in the other three strains, perhaps due to the physiological opposition of D2 activity by D1 activity in this mixed D1/D2 agonist. On the other hand, quinpirole failed to significantly alter locomotor activity in CBA/J mice, whereas quinpirole decreased activity in three other strains of mice. Moreover, apomorphine and the selective D1 agonist SKF 38393 were essentially equipotent and equieffective in increasing locomotor activity in CBA/J and other strains of mice. Taken together, the present results are consistent with the interpretation that CBA/J mice are deficient in D2, but not D1, dopamine receptor function.

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