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The duration of the effects of a single administration of dopamine antagonists on ambulatory activity and motor coordination

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Summary. Pimozide, cis(Z)-flupenthixol, SCH 23390 and sulpiride were administered to male rats. Each subject received a single drug injection, and tests for ambulatory activity and motor coordination were performed 1, 24, 48 and 72 hrs later. All drugs reduced ambulatory activity at the test 1 hr postinjection. Pimozide and SCH 23390 continued to reduce ambulatory activity at the test 24 hrs after injection. All drugs impaired motor coordination 1 hr after injection and, with the exception of SCH 23390, were also effective at the 24 hrs test. Flupenthixol, 2 mg/kg, continued to impair motor coordination also at the 48 hrs test. These data show that effects of dopamine antagonists on motor functions may persist for much longer than is generally believed. That should be important to take into account in experimental designs where repeated drug administration is employed.

Keywords: Neuroleptics, ambulatory activity, motor coordination, duration of effect.

Introduction

Dopamine antagonists are widely used in behavioral studies. One of their most prominent effects is to reduce ambulatory activity. In addition, motor coordination is frequently impaired and catalepsy may be produced as well (reviewed in LeMoal and Simon, 1991). It is common to employ repeated measures designs when drug effects on behavior are analyzed, in such a way that the same animal is treated with the same or different doses of an antagonist or with different antagonists on repeated occasions. The interval between successive drug treatments varies between studies, but may be as short as 24 or 48 hrs (e.g. Acquas and Di Chiara, 1994; Spyraki et al., 1985). In the course of studies of behavioral effects of dopamine antagonists (Ågmo and Fernández, 1989; Ågmo and Picker, 1990; Ågmo et al., 1993), we accidentally observed that some effects on motor functions persisted for 24 hrs or more. It must be noted that other behavioral effects of dopamine antagonists, e.g.

inhibition of intracranial self-stimulation or blockade of amphetamine-induced stereotypies, have been shown to be absent 24 hrs after drug administration (Janssen et al., 1968). This would suggest that the duration of effect cannot be generalized from one behavior to another. Information concerning the duration of motor effects seems to be important, because if our accidental observations were confirmed, drug effects could be carried over from one treatment session to the next, thus confounding results of repeated measures experiments.

Despite an exhaustive search of the Medline 1966–1996 database we could not find any study where the duration of action of dopamine antagonists on motor functions had been studied for more than a few hrs. The purpose of the following experiments was to determine the duration of the effect of some commonly employed dopaminergic antagonists on ambulatory activity and motor coordination using a longer time span than in previously published studies.

Materials and methods

Animals and housing

Male Wistar rats (350–450 g) from the animal facilities of the Faculty of Medicine, National Autonomous University of Mexico were housed 2 per cage under a reversed light/dark cycle (12/12 hrs, lights off 0900) with constant access to tap water and commercial rodent pellets (Purina). The temperature in the animal quarters was maintained at 22 ± 1°C and humidity was not controlled.

The experiments reported herein were performed according with the Guide for the Care and Use of Laboratory animals as adopted and promulgated by the National Institutes of Health of the United States of America and in agreement with applicable local laws.

Apparatus and procedure

Ambulatory activity was quantified in cylindrical steel cages (diameter 60 cm) equipped with 6 infrared photocells located at regular intervals on the circumference 2.5 cm above the grid floor. The counters were activated by photobeam interruptions longer than 250 msec. This means that rapid movements, such as tail flicks or scratching, were not recorded. The photocell counts thus mainly represent ambulatory activity. Before experiments, the subjects were familiarized to the activity cages during three sessions of 10 min each separated by 48 hrs. There is a considerable reduction of ambulatory activity between the 1st and 2nd session and a further reduction is observed on the 3rd session. Then, however, ambulatory activity stays stable. Activity tests lasted 10 min. Motor coordination was evaluated on a rotarod. A cylinder (diameter 16 cm) made of steel with a specially prepared rough surface rotated at 11 rpm. Whenever an animal fell down from the cylinder, it was immediately replaced on it. The number of falls during a 3 min test constituted the measure of motor incoordination. All subjects were trained to walk on the cylinder as described elsewhere (Ågmo et al., 1987) a few days before experiments. Tests were performed between the 3rd and the 7th hr of the dark phase of the light/dark cycle. Different groups of animals were used for tests of ambulatory activity and motor coordination. All animals were drug naive.

Drugs

The mixed dopamine D1/D2 antagonists pimozide (Janssen, Beerse, Belgium) and cis(Z)-flupenthixol (Lundbeck, Copenhagen, Denmark) were dissolved in dilute acid (1 drop of glacial acetic acid added to 1 ml of physiological saline) and physiological saline, respectively. The dopamine D1 antagonist SCH 23390 hydrogen maleate ((R)-(+)-7-chloro-8-hydroxy-3methy-2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine, Schering Corporation, Bloomfield, NJ, USA) was suspended in physiological saline with a drop of Tween 80 while the D2 antagonist sulpiride (Delagrange International, Paris. France) was dissolved in physiological saline heated to about 70°C to which a few drops of 1 M HCl was added until a clear solution was obtained. This was then cooled to body temperature before injection. All drugs were administered intraperitoneally in a volume of 5 ml/kg body weight (pimozide, sulpiride) or 1 ml/kg (flupenthixol, SCH 23390). Controls received a similar injection of the appropriate vehicle. The drugs were injected 24 hrs after the first experimental test for ambulatory activity or motor coordination. One hr later a second test was performed. Additional tests were performed 24, 48 and 72 hrs after drug injection.

Statistical analyses

Ambulatory activity data were analyzed with two-factor ANOVAs for repeated measures on one factor. The between groups factor was dose of drug and the within groups factor was time after injection. In case of significant interaction, tests for simple main effects of dose at each test were made. A posteriori comparisons were performed with Tukey's HSD test. Data from the motor coordination test were analyzed by a separate Kruskal-Wallis ANOVA for each time after injection. In case of significance, all groups were compared to control with the Mann-Whitney U-test. Parametric tests could not be used because of non-normal distribution of the data (most controls had a value of 0) and non-homogeneous error variances as determined by Hartley's F_{max} test. All probabilities given are two-tailed.

Results

Ambulatory activity

ANOVA showed significant effects of dose and of time after injection for the four drugs employed (all $Ps < 0.01$). The interaction dose × time after injection was also significant in every analysis ($Ps < 0.01$). Therefore, tests for simple main effect of dose were performed for each test. As can be seen in Fig. 1, pimozide reduced ambulatory activity at the test 1 hr after drug administration at the doses of 1 and 2 mg/kg. The largest dose was also effective at 24 and 48 hrs postinjection. Flupenthixol had a strong inhibitory action on ambulatory activity at 1 hr postinjection when administered at the doses of 1 and 2 mg/kg. Indeed, the 2 mg/kg group was almost totally inactive. Nevertheless, this effect had completely disappeared at the test 24 hrs after injection. SCH 23390 also reduced ambulatory activity 1 hr after injection. All doses were effective, and there did not seem to be any dose-dependency. The largest reduction of activity was observed after 0.5 mg/kg, but there was no statistically significant difference between doses. The following day, the lowest dose, 0.25 mg/kg, continued to reduce activity whereas the other doses were ineffective. At 48 hrs postinjection, there was no effect of SCH 23390. With regard to sulpiride, only the largest dose, 120 mg/kg, significantly reduced ambulatory activity, and only at the test 1 hr postinjection. Data are shown in Fig. 1.

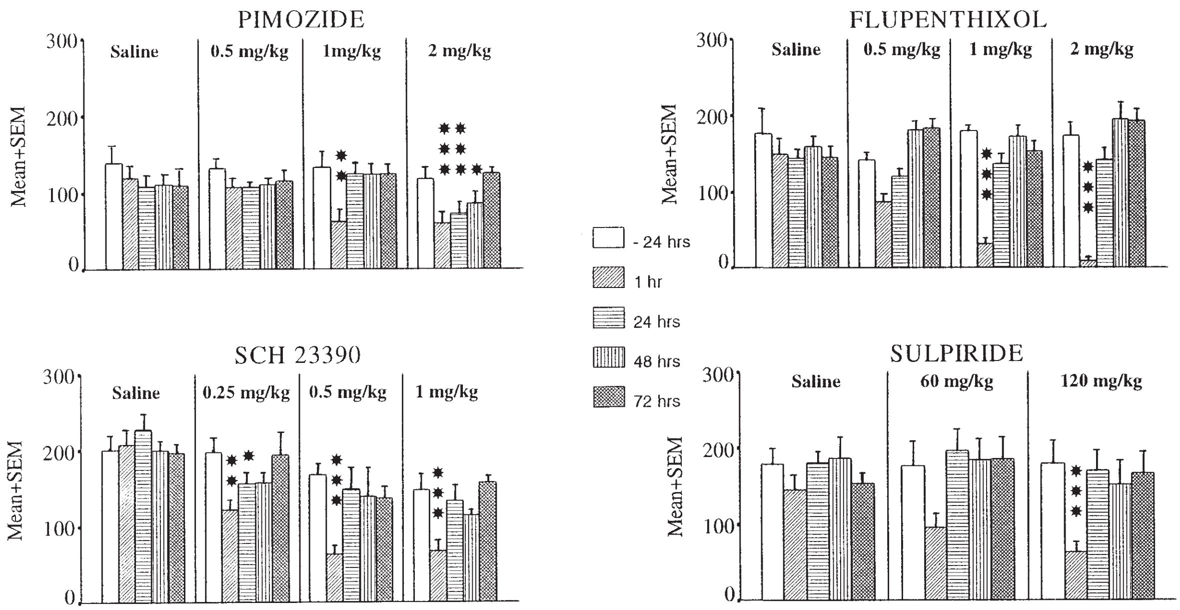

Fig. 1. Ambulatory activity, expressed as the mean + SEM number of beam interruptions during a 10 min test, in male rats treated with varying doses of pimozide, cis(Z)-flupenthixol, SCH 23390 or sulpiride. There were 8 to 11 animals per dose, depending on the drug. *, different from control, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Motor coordination

Pimozide, 2 mg/kg, impaired motor coordination at the test performed 1 hr after injection. At the test 24 hrs postinjection, this dose as well as the 1 mg/kg dose produced motor impairment. The effect of the largest dose appeared to last for at least 72 hrs, but there was no statistically significant difference between this group and controls at the 48 and 72 hrs tests (Fig. 2). Flupenthixol, at doses of 1 and 2 mg/kg, impaired motor coordination at 1 hr postinjection, and this effect lasted for 48 hrs for the 2 mg/kg dose and 24 hrs for the dose of 1 mg/kg. Motor coordination was impaired by SCH 23390, 0.5 and 1 mg/kg, at the test 1 hr postinjection. No effect was found at 24 hrs or later. The 120 mg/kg dose of sulpiride impaired motor coordination at the test 1 hr after injection as well as at the test 24 hrs postinjection. Data are illustrated in Fig. 2.

Discussion

A crucial question determining the relevance of the present data is whether the doses of the dopamine antagonists employed here are within the range that is normally used in behavioral studies. This seems indeed to be the case for the lowest dose of each drug and in the case of pimozide and sulpiride also for the largest doses used (see e.g. Fujiwara, 1992; Hoffman and Beninger, 1985; Koek and Colpaert, 1993; Morgenstern and Fink, 1985; Papa et al., 1993).

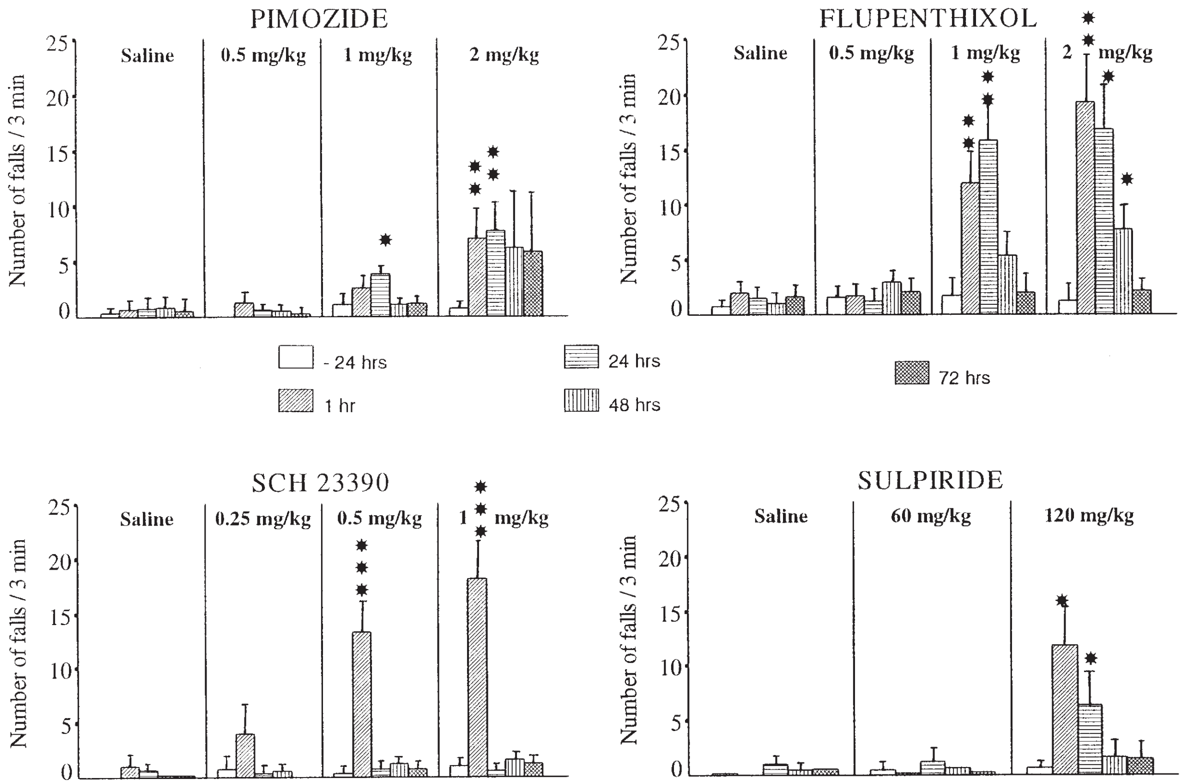

Fig. 2. Motor coordination, as evaluated on a rotarod, in male rats treated with several doses of pimozide, cis(Z)-flupenthixol, SCH 23390 or sulpiride. Data are mean + SEM. There were 10 animals per dose and drug. *, different from control, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

The present data show that pimozide, at the dose of 2 mg/kg, reduced ambulatory activity for 48 hrs. Pimozide has been reported to have a half-life of about 5.6 hrs in the brain and plasma of rats (Janssen and Allewijn, 1968). This means that the drug was behaviorally effective at a time when its brain concentration was very low (Soudjin and van Wijngaarden, 1972). One explanation for this is that some metabolite of the drug is pharmacologically active. This seems unlikely, however, because short-term pharmacological effect has been reported to be correlated with unchanged pimozide concentrations and not with metabolites (Soudjin and van Wijngaarden, 1972).

The other mixed D1/D2 antagonist, flupenthixol, differed from pimozide in the way that ambulatory activity was reduced only at the test 1 hr postinjection. Cis(Z)-flupenthixol has a half-life in plasma and brain of about 16 hrs, with peak levels attained at about 4 hrs postinjection (Jörgensen et al., 1969). Thus, its half-life is longer than that of pimozide, yet the effects on ambulatory activity are of shorter duration. This latter fact suggests that the reduced activity observed 24 hrs after pimozide injection was not a consequence of some hypothetical learned inhibition. It has, in fact, been reported that the locomotor reducing effect of SCH 23390 but not that of the

D2 antagonist metoclopramide can be conditioned (Mazurski and Beninger, 1991). Insofar as flupenthixol is more potent at the D1 receptor than pimozide (Hyttel and Arnt, 1987), its effects should, in principle, be more easily conditioned than those of the latter drug. The fact that flupenthixol was inactive at the second postinjection test reinforces the notion that conditioning is not important for the prolonged effects of pimozide or any other drug used. Nevertheless, we performed an additional experiment in order to rule out the possibility that long-lasting drug effects were due to some kind of learning or modified motivation brought about by repeated testing. Thus, rats were given a test 24 before the injection of either saline or pimozide, 1 or 2 mg/kg. The animals were tested once 48 hrs after the injection. As can be seen in Fig. 3, 1 mg/kg was ineffective whereas 2 mg/kg reduced locomotor activity and impaired motor coordination at this test. These data are almost identical to those obtained in the main experiment and show that repeated testing

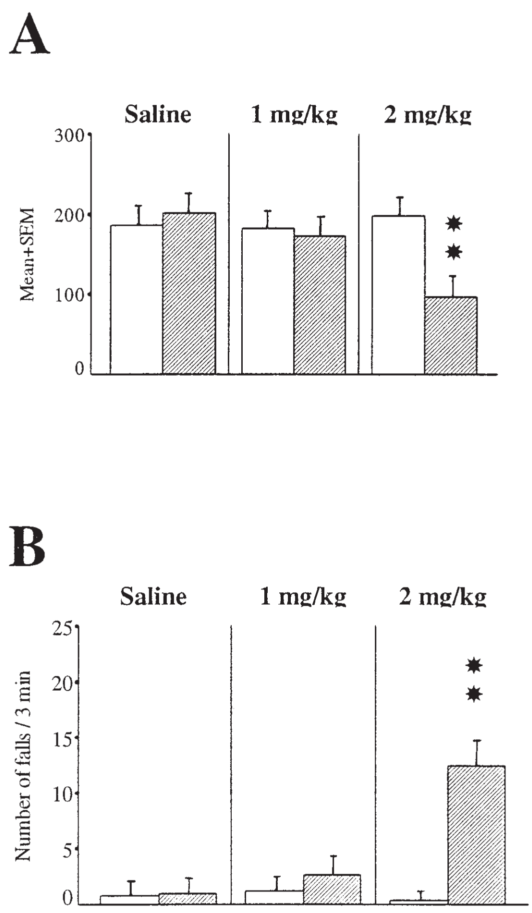

Fig. 3. **A** Ambulatory activity and **B** motor coordination in male rats given saline or varying doses of pimozide. White bars, test performed 24 hrs before drug administration; striped bars, test 48 hrs after drug administration. Data are mean + SEM. There were 10 animals per dose. **, different from control, $P < 0.01$

cannot account for the long duration of drug effects observed in the present studies.

It is also unlikely that drug effects such as reduced food and/or water consumption could account for the impaired motor behavior observed in our experiments. In many behavioral studies animals deprived of food and water are used without showing any signs of motor impairment. If anything, food deprivation increases ambulatory activity (File and Day, 1973; Mabry and Campbell, 1975).

The dopamine D1 antagonist SCH 23390 reduced ambulatory activity 1 hr postinjection. SCH 23390 was also active on ambulatory activity at the 24 hrs test. It has been reported that the peak level of this drug in plasma is attained 1 hr after injection, whereas brain concentrations continues to increase for at least 3 hrs (Schulz et al., 1985). This is in contrast to pimozide and flupenthixol where brain and plasma concentrations peak and decline with similar time courses (Janssen and Allewijn, 1968; Jörgensen et al., 1969). It appears, therefore, that SCH 23390 persistently binds to dopamine receptors within at least certain regions of the CNS, and this could perhaps be responsible for the prolonged behavioral effects observed here. There are data showing that a local injection of SCH 23390 into the medial prefrontal cortex, that does not by itself modify ambulatory activity, reduces the locomotor effect of intraaccumbens amphetamine 2 days later (Vezina et al., 1994). Whether such long-lasting actions can explain our results is not known.

Sulpiride affected ambulatory activity only at the test 1 hr postinjection, where the 120 mg/kg dose produced a strong reduction. No effect was observed on later tests. The half-life of sulpiride has been estimated to be about 2.5 hrs in plasma of rats (Kamizono et al., 1993), which is much shorter than that of most other drugs. This coincides with the absence of an effect on ambulatory activity 24 hrs after injection. However, flupenthixol, which has a much longer half-life, was also inactive at that test. It appears, therefore, that a drug's half-life is not a good predictor of the duration of at least some of its behavioral actions. This notion is reinforced by the fact that pimozide, which also has a shorter half-life than flupenthixol (see above), reduced ambulatory activity longer than that drug. Moreover, our data suggest that it is not the receptor type, D1 or D2, that determines the duration of action, but the specific drug employed. Flupenthixol is almost as active at the D1 receptor as SCH 23390 (Hyttel and Arnt, 1987), yet it was inactive at 24 hrs. On the other hand, pimozide binds to the D2 receptor more readily than to the D1 (Hyttel, 1983), but its actions on ambulatory activity are similar to those of SCH 23390 and not to those of sulpiride.

When motor coordination was analyzed, it was found that all drugs except SCH 23390 were effective 24 hrs postinjection. Even pimozide inhibited motor coordination at a dose of 1 mg/kg at the 24 hrs test. This dose was ineffective at the 1 hr test, and had no effect on ambulatory activity at the 24 hrs test. Flupenthixol, 1 and 2 mg/kg, as well as sulpiride, 120 mg/kg, impaired motor coordination at this time-point without having any effect on ambulatory activity. On the other hand, SCH 23390 reduced ambulatory activity at 24 hrs postinjection while having no effect on motor coordination. Thus, effects on ambulatory activity are independent from effects on motor coordination. These results suggest that there is a dissociation between the effects of dopamine antagonists on different motor functions. At the moment, there is no clear explanation available for these observations. One possibility is that the different structures involved in the control of ambulatory activity and motor coordination are differentially sensitive to long-term effects of dopamine antagonists. It is generally believed that ambulatory activity depends on dopaminergic function within the nucleus accumbens while motor coordination is mostly dependent on the nigro-striatal dopamine system (Scheel-Krüger and Willner, 1991). There may be functional differences between these systems. Another possibility is that acute treatment produces depolarization block of some dopaminergic neurons but not of others, and that this block outlasts the presence of drug. Acute depolarization block in the mesolimbic as well as in the nigrostriatal system has been observed in some experimental situations (Henry et al., 1992; Hollerman et al., 1992; Rompré and Wise, 1992). However, this explanation is entirely speculative. Nevertheless, present data show that intervals between repeated drug administrations need to be long if cumulation of effects on motor functions are to be avoided, particularly if the doses employed are large.

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