Role of striatal dopamine D₂ receptors in the paw test, an animal model for the therapeutic efficacy and extrapyramidal side effects of neuroleptic drugs

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Abstract

The effect of administration of the D₂ antagonist sulpiride in three striatal areas (dorsal striatum, DS; nucleus accumbens, ACC; olfactory tubercle; OT) was studied in the so-called paw test. In the paw test two parameters are measured (the hindlimb retraction time (HRT) and the forelimb retraction time (FRT)) that model the therapeutic efficacy and the extrapyramidal side effects of neuroleptics, respectively. Sulpiride significantly enhanced the HRT in each of the three structures. Identical doses of sulpiride administered in the three structures produced similar effects. The FRT was enhanced after administration of sulpiride in the DS and in the ACC. The minimal effective dose was lower for the DS. Administration of sulpiride in the OT did not affect the FRT. The effects on the FRT were very slow in onset (strong effects 4 h or more following administration of sulpiride), especially in comparison to the rapid effect on FRT following systemic administration of classical neuroleptics. To analyze this slowness of effect, two additional experiments were performed: first, the inter-trial time was changed so that it was identical to that used in systemic studies; second, sulpiride was administered simultaneously in the DS and the ACC. Neither experiment produced an earlier effect on the FRT. The present data provide additional evidence for the theory that regional selectivity of drugs determines their propensity to induce extrapyramidal side effects. However, the data also suggest that the generally held view that the dorsal striatum is solely responsible for the extrapyramidal side effects of neuroleptic drugs is too simple.

Keywords: Neuroleptic; Paw test; Neostriatum; Nucleus accumbens; Olfactory tubercle; Sulpiride; D₂ receptor

1. Introduction

A large number of studies have shown that classical and atypical neuroleptics can be discerned with respect to their regional selectivity. Thus, chronic administration of atypical neuroleptics attenuates spontaneous firing in dopamine A10, but not A9 cells, while classical neuroleptics affect both A10 and A9 cells [4,23]. Atypical neuroleptics selectively block locomotor activity induced by dopamine in the nucleus accumbens (ACC) as compared to locomotor activity induced by dopamine in the dorsal striatum [7] (DS). These in vivo behavioural and electrophysiological findings are supported by pharmacological studies showing that atypical neuroleptics affect spiperone binding in the ACC and/or OT stronger than in the DS, while the reverse is true for classical neuroleptics [1,16,17], but see [18,21]. Taken together, most studies suggest that drugs that selectively block the striatal A10 system (ACC and OT) have a low propensity to induce EPS (viz. atypical neuroleptics).

On the other hand, regional selectivity of neuroleptics is also found within the A10 system: classical neuroleptics block locomotor activity elicited from the ACC stronger than that elicited from the OT, while the reverse is true for atypical neuroleptics [5,6]. The latter studies suggest that neuroleptics not only show selectivity for the dopamine A9 system vs. the dopamine A10 systems, but show selectivity within the A10 system as well. To further characterize the role of the DS, the ACC and the OT, the present study further analyses the role of these structures in the paw test, an animal model with predictive validity for both the antipsy-
chotic potential (the hindlimb retraction time) and the extrapyramidal side effects (the forelimb retraction time) of drugs [9-11]. The effects of intracerebral administration of sulpiride, a D₂ selective antagonist and neuroleptic, are determined.

The results show that sulpiride affects the hindlimb retraction time after administration in all three areas, whereas the forelimb retraction time is especially affected after administration of sulpiride into the DS. However, the effects of sulpiride on the forelimb retraction time were slow in onset, in comparison with systemic administration of neuroleptics [11]. To analyze this slowness of effect, two additional experiments were performed: first, the inter-trial time was changed so that it was identical to that used in systemic studies [11]; second, sulpiride was administered simultaneously in the DS and the ACC, two areas involved in the effects of sulpiride upon the forelimb retraction time. However, the results show that these additional experiments did not change the slow onset of the effect on the forelimb retraction time.

2. Materials and methods

2.1. Subjects and surgery

Naive, male Wistar rats (n = 7-12 animals per group; Central Animal Laboratory, Nijmegen) were used. Rats weighing between 180-220 g were anaesthetized with sodium pentobarbital (Narcovet, 60 mg/kg, i.p.) and placed in a stereotaxic apparatus. Guide cannulas (outer diameter: 0.65 mm; inner diameter: 0.30 mm) were bilaterally implanted into the DS (ant. 9.4, vert. 1.0, lat. 2.5), the ACC (ant. 9.8, vert. 2.7, lat. 1.2) and the OT (ant. 8.8, vert. 1.1, lat. 2.2) [14]. Cannulas (5 mm length) directed at the ACC were angled 10 degrees laterally, while cannulas directed at the DS (4 mm length) and OT (6 mm length) were not angled. Cannulas were fixed onto the skull with dental cement (Durelon, ESFE; carboxylate cement) aided by the attachment of two screws. In an additional experiment, every animal received four cannulas: two aimed bilaterally at the ACC (cannulas implanted at ant. 9.8 mm, vert. 0.0 mm, lat. 1.0 mm, no lateral angle), and two the DS (cannulas implanted at ant. 9.4, vert. 0.0 mm, lat. 2.5, vertical angle 10°). After surgery the rats were housed individually in the original stockroom and allowed recovery from operation for at least one week. Rats were kept on a 12-h day/night cycle with lights on at 07.00 h. Food and water were available ad libitum.

2.2. Experimental test design

At the day of the experiment, rats received bilateral injections of DL-sulpiride (Sigma; dissolved in distilled water and a drop of acetic acid after which the pH was adjusted to 6-7) in the DS, the ACC or the OT at t = 0 min. Injections were given by means of a 5 μl syringe with a 0.25 mm needle that extended into the brain tissue below the tip of the permanent embedded cannula (0.5 mm for DS; 1.0 mm for ACC; 1.5 mm for OT). The volume was 0.5 μl injected over a 10 s period, and the needle was left in situ for another 10 s to minimise diffusion along the needle tract. Then, the inner cannula was re-inserted. Earlier studies have shown that this procedure leads to highly localized injections [20]. In the experiment in which rats with four cannulas were used drugs were first injected into the ACC, immediately followed by bilateral injections into the DS. The paw test was performed at t = 30 and repeated at t = 60, 90, 120, 180, 240, 300 and 360 min for each rat (in one additional experiment, the inter-trial time was limited to 10 min, so that the paw test was performed at t = 30, 40, 50, 60, 70, 80, 90 and 100 min).

Testing occurred between 09.00 and 17.00 h. Animals were only used for a single experiment. In the paw test a rat was placed on a Perspex platform, measuring 30 by 30 cm with a height of 20 cm and having four holes, two holes of 5 cm diameter for the hindlimbs and two holes of 4 cm for the forelimbs [11]. The rat was placed on the platform by positioning first the hind- and then the forelimbs in the holes. Two variables were measured in this test, namely the time it takes the animal to retract its first hindlimb (hindlimb retraction time; HRT) and the time it takes the animal to retract its first forelimb (forelimb retraction time; FRT) with a minimum time of 1 s and a maximum time of 30 s. Since only robust effects are considered to be important, the window (1 to 30 s) was chosen so that significant increases and significant decreases could be easily detected.

2.3. Data representation and statistics

The data are presented as the median value of the scores per group (n = 7-11 rats) together with the standard error of the median, since the data showed no normal distribution. The individual scores were transformed to ranks and statistically analyzed, using the SAS statistical package. Drug effects were analyzed with a repeated nonparametric analysis of variance (ANOVA) with factors drug and time. Since we are only interested in drug effects, a significant effect on the factor time will not be mentioned. For dose-dependent effects, identical statistical analysis was performed, but now with factors dose and time. Finally, in the last experiment, a repeated nonparametric ANOVA was performed on the rank-transformed data with factors interval (duration) and (number of) trials (see section 3). After the experiments rats were sacrificed,
and brains were dissected. Brain sections were microscopically analyzed. Only data from subjects with injections made into the desired sites (Fig. 1) were further analyzed.

3. Results

3.1. Effects of intra-striatal administration of sulpiride on the HRT

In the DS, 25 ng sulpiride significantly enhanced the HRT ($F_{1,7} = 37.5$, $P < 0.001$; Fig. 2). A higher dose of sulpiride (100 ng) produced significant greater effects than the dose of 25 ng ($F_{1,18} = 9.8$, $P < 0.01$; Fig. 2). This dose of 100 ng induced an optimum effect since a higher dose of sulpiride (400 ng) produced an effect that was significantly smaller compared with that of 100 ng in the DS ($F_{1,7} = 7.6$, $P < 0.05$; data not shown). In the ACC, 25 ng sulpiride significantly enhanced the HRT ($F_{1,14} = 179.0$, $P < 0.001$; Fig. 2). A higher dose of sulpiride (100 ng) produced significant greater effects than the dose of 25 ng ($F_{1,11} = 10.7$, $P < 0.01$; Fig. 2). Further enhancing the dose to 400 ng did not produce effects different from those of 100 ng (data not shown). In the OT, 25 ng sulpiride significantly enhanced the HRT ($F_{1,14} = 6.2$, $P < 0.05$; Fig. 2). A higher dose of sulpiride (100 ng) produced significant greater effects than the dose of 25 ng ($F_{1,15} = 13.2$, $P < 0.01$; Fig. 2). Further enhancing the dose to 400 ng did not produce effects different from those of 100 ng (data not shown).

Sulpiride (200 ng) administration in both the DS and the ACC did not differ from sulpiride administration (200 ng) in the DS alone, viz. the ‘control’ group that produced the strongest effects (Fig. 4A). The effects of 200 ng sulpiride in the last trial in our normal paradigm (testing at 30, 60, 90,...,360 min) were not different from the effects in the last trial in the paradigm with the short inter-trial interval of 10 min (testing at 30, 40,..., 100 min) both for the DS and the ACC (data not shown).

3.2. Effects of intra-striatal administration of sulpiride on the FRT

The lowest dose of sulpiride tested (25 ng) did not affect the FRT in any of the three areas (data not shown). Higher doses of sulpiride (100–400 ng) produced significant effects after administration in the DS (100 ng: $F_{1,19} = 65.3$, $P < 0.001$; 400 ng: $F_{1,17} = 50.7$, $P < 0.001$; Fig. 3). In the ACC, sulpiride in a dose of 100 ng did not affect the FRT (Fig. 3). Further enhancing the dose of sulpiride in the ACC to 400 ng produced a significant effect compared with controls ($F_{1,16} = 9.2$, $P < 0.01$; Fig. 3). In the OT, 100 or 400 ng of sulpiride induced no significant effects (data not shown). Sulpiride (200 ng) administration in both the DS and the ACC did not differ from sulpiride administration in the DS alone, viz. the ‘control’ group that produced the strongest effects (Fig. 4B).

In contrast to what was expected (see Introduction), the effects of 200 ng sulpiride in the last trial in our normal paradigm (testing at 30, 60, 90,...,360 min) were significantly higher than the effects in the last trial of the paradigm with the short inter-trial interval of 10 min (testing at 30, 40,..., 100 min; Fig. 5): an interaction between interval and trials was found for both for the DS ($F_{7,98} = 5.66$, $P < 0.001$) and the ACC ($F_{7,91} = 3.75$, $P < 0.01$; Fig. 5).

Fig. 1. The areas in which the injection places were located for the dorsal striatum (A), the nucleus accumbens (B) and the olfactory tubercle (C) according to the atlas of Paxinos and Watson [19].
4. Discussion

4.1. The paw test

The present results show that intracerebral injections of sulpiride can affect the parameters measured in the paw test. Before the implication of these findings can be discussed it is relevant to recall the validity of the paw test parameters. As has been discussed at length in other papers [9–11] the paw test measures both forelimb retraction time (FRT) and hindlimb retraction time (HRT). The HRT represents an animal model for the therapeutic effects of neuroleptic drugs: [1] All tested neuroleptics increase HRT [9,11]; [2] No false positives or negatives have so far been identified [9,11]; [3] There is a correlation between the potency of neuroleptics to affect HRT and to induce therapeutic effects in the clinic [9]; [4] Anticholinergic drugs do not affect the effects of neuroleptic drugs [10]; [5] Chronic treatment does not lead to tolerance towards the effects of neuroleptics on HRT [10] and [6] Benzodiazepines enhance the effects of neuroleptics [9]. Likewise, there is strong evidence that FRT represents a good animal model for the extrapyramidal side effects: [1] Only classical neuroleptics enhance FRT [9,11]; [2] Anticholinergic drugs antagonise the effects of neuroleptics on FRT [10] and [3] Chronic treatment reduces the effects of neuroleptics on FRT [10]. Taken these data together, it seems that the paw test represents a valid model for both the therapeutic and the extrapyramidal side effects of neuroleptic drugs.

4.2. Effects of intra-striatal administration of sulpiride on the HRT

Systemic administration of both classical and atypical neuroleptics produces strong effects on the HRT in the paw test [11]. The present study examined (a.o.) to what extent administration of the D<sub>2</sub> antagonist sulpiride in distinct striatal areas could mimic such an effect. The present data show that administration of sulpiride into the DS, the ACC and the OT all produced strong effects on the HRT, that were largely comparable from a quantitative point of view. Thus, blockade of D<sub>2</sub> receptors in all three striatal areas may be responsible for the effects observed after systemic administration of D<sub>2</sub> antagonists. The effects of sulpiride administered in these areas are considered to

Fig. 2. The effects of local application of sulpiride in three striatal areas on the hindlimb retraction time (HRT). Every animal was repeatedly tested in the paw test at the time points shown. Median values±S.E.M. of the HRT are given. (●) Controls; (○) 25 ng sulpiride; (□) 100 ng sulpiride. A: dorsal striatum. B: nucleus accumbens. C: olfactory tubercle.
4.3. Effects of intra-striatal administration of sulpiride on the FRT

Systemic administration of classical, but not atypical, neuroleptics produces strong effects on the FRT in the paw test [11]. The present study examined to what extent intra-striatal administration of a D$_2$ antagonist could mimic such an effect. The present data show that administration of sulpiride into the DS and to a lesser extent into the ACC produced effects on the FRT. Thus, striatal D$_2$ receptors, especially in the DS, but

be specific for the area studied, since sulpiride diffuses hardly following intracerebral administration [2,15]. On the other hand, one study found evidence that (−)-sulpiride can diffuse from the DS to the ACC [22]. However, it has to be noted that the dose of sulpiride used in this latter study (40 μg) was at least 100-fold higher than the highest dose used in the earlier-mentioned studies as well as in the present study [22].
also in the ACC, may be responsible for the effects in the paw test following systemic administration of D₂ antagonists. However, unlike the effects seen after systemic administration of D₂ antagonists, sulpiride administered in the DS or ACC produced its effects on the FRT only after several hours of repeated testing. Similar delayed effects of sulpiride were reported on catalepsy [12], which is like the FRT an animal model for extrapyramidal side effects. The delayed effect on the FRT was not due to the large inter-trial interval (30 or 60 min) that was used in the present study; a control experiment using the 10 min inter-trial interval that produces strong effects on FRT following systemic injections of classical neuroleptics [11], did not produce a faster increase in FRT (Fig. 5B). In fact, the 10 min interval produced significantly smaller effects compared with our normal paradigm (30, 60..., 360 min testing), showing that the strong increase in FRT found in the present study, is not a direct consequence of repeated testing. Alternatively, it is theoretically possible that this slowness of onset was due to diffusion of sulpiride to the DS and/or the ACC. In the present study, however, simultaneous administration of sulpiride in both the DS and the ACC produced effects with similar slow onset (Fig. 5). Therefore, it is unlikely that the tardy effects of sulpiride were due to the slow diffusion of sulpiride to these two structures. The most logical explanation is that the very slow diffusion of sulpiride in the distinct areas itself and, thus, the amount of D₂ receptors blocked within these areas caused this slow onset effect. Indeed, extrapyramidal side effects, for which the FRT is a model (see below), are known to require the blockade of about 80% of D₂ receptors [13].

Given the predictive validity of the paw test showing that the HRT is a model for the therapeutic efficacy of neuroleptics and that the FRT is a model for the extrapyramidal side effects of neuroleptics (see above), the present study may contribute to our understanding of the role of regional selectivity in the EPS profile of neuroleptics. In contrast to the general hypothesis that neuroleptics produce their therapeutic effects by blocking A₁₀ dopaminergic cells and produce extrapyramidal side effects by blocking A₉ dopaminergic cells, our data suggest a more complicated picture. The fact that sulpiride administered in the DS, the ACC and the OT is equipotent in enhancing the HRT strongly suggests that all three regions equipotently contribute to the antipsychotic efficacy of neuroleptics. On the other hand, the finding that sulpiride administered in the DS is more potent than sulpiride administered in the ACC in enhancing the FRT strongly suggests that neuroleptics with selectivity for the A₉ system over the A₁₀ system have a relatively high propensity to induce EPS, and that the reverse holds true for neuroleptics with selectivity for the A₁₀ system (see Introduction). Moreover, since sulpiride administered in the OT is still less potent in inducing an increase in FRT than sulpiride administered in the ACC, these data also explain why neuroleptics with selectivity for the OT over the ACC have a lower propensity to induce EPS than neuroleptics with selectivity for the ACC over the OT [5,6]. Finally, it should be noted that extra-striatal areas may also play an important role in the therapeutic effects or side effects of neuroleptics, such as the hippocampus [3] and the prefrontal cortex [8].
References


