Contralateral turning elicited by unilateral stimulation of dopamine D$_2$ and D$_1$ receptors in the nucleus accumbens of rats is due to stimulation of these receptors in the shell, but not the core, of this nucleus

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Abstract The goal of this study was to determine whether dopamine D$_2$ and/or D$_1$ receptors in the shell and the core of the nucleus accumbens of rats have a differential role in turning behaviour. Unilateral injection of a mixture of the dopamine D$_2$ receptor agonist quinpirole (10 µg) and the dopamine D$_1$ receptor agonist 1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol (SKF 38393, 5 µg) into the shell of the nucleus accumbens produced contralateral turning, when doses which per se were ineffective were injected. This effect was far greater than that found after similar injections into the core of the nucleus accumbens. The effect elicited from the shell was significantly attenuated by prior administration of either the dopamine D$_2$ receptor antagonist l-sulpiride (25 ng/0.5 µl) or the dopamine D$_1$ receptor antagonist (8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol (SCH 23390, 0.5 µg/0.5 µl) into the same region. These data together with the fact that l-sulpiride is known to be a valid tool to differentiate the involvement of distinct regions within the shell underlie the conclusion that dopamine D$_2$ and D$_1$ receptors in the shell, but not the core, of the nucleus accumbens play a critical role in the contralateral turning induced by unilateral injection of dopamine receptor agonists into this nucleus. The results are discussed in view of the known output pathways of the shell.

Key words SKF 38393 • Quinpirole • SCH 23390 • l-Sulpiride • Nucleus accumbens shell • Nucleus accumbens core • Turning behaviour • Dopamine D$_1$ and D$_2$ receptors • Rat

Introduction

Unilateral manipulation of neural activity in the nucleus accumbens is known to elicit unilateral turning in freely moving rodents (Colie and Wise 1991; Messier et al. 1991a, b; McKenzie et al. 1991; Saigusa et al. 1993). Recently, it has become evident that the nucleus accumbens is a heterogeneous structure. At least two different parts can be discerned: the shell and the core (Voorn et al. 1986; Groenewegen et al. 1987; Heimer et al. 1991; Zahm and Brog 1992; Brog et al. 1993; Meredith et al. 1993; Jongen-Relo et al. 1994). The shell and the core appear to be innervated by different sets of dopaminergic neurons (Voorn et al. 1986; Zahm 1991, 1992). The shell contains a richer dopamine plexus than the core (Voorn et al. 1986), and the concentration of dopamine in the shell is higher than that in the core (Deutch and Cameron 1992). The shell is less vulnerable to the neurotoxic 6-hydroxydopamine than the core (Zahm 1991). Furthermore, the dopamine D$_2$ binding appears to be lower in the shell than in the core, whereas the dopamine D$_1$ binding in the rostral areas of the nucleus accumbens appears to be higher in the shell than in the core (Bardo and Hammer 1991; Jongen-Relo et al. 1992). These data suggest that functional differences in the neurotransmission occur in the two domains. Indeed, recent studies on oral behaviour have shown that especially the shell, but not the core, plays a critical role in the expression of oral behaviour (Cools et al. 1993, 1995; Prinssen et al. 1994).

Given the differences in the dopamine neurotransmission between the shell and the core, the differential involvement of these two domains in oral behaviour, and the role of the nucleus accumbens in turning behaviour, the question arose whether dopamine D$_2$ and/or D$_1$ receptors in the shell and core have also a differential role in turning behaviour. Since previous studies have shown that intra-accumbens administration of a
The rats were placed individually in a circular Perspex chamber (60 cm diameter and 30 cm high) 1 h before the start of the experiment. To allow detailed observation of the turning behaviour, the limb stepping patterns and the type of spinal curvature, a mirror was mounted underneath the chamber at an angle of 30° and the image was recorded on videotape for off-line analysis. Contralateral and ipsilateral turnings (defined as complete 360° turns) were counted visually by a trained observer who had no prior knowledge of the drug treatment. Previously, it has been found that intracerebral administration of L-sulpiride and SCH 23390 can produce dragging of the hindlimbs and a reduced ability to move the head independently of the torso, respectively (Saigusa et al. 1995). Accordingly, particular attention was paid to the display of these phenomena. The behaviour was analyzed during consecutive 5-min periods for 120 min, immediately starting after the injection.

Materials and methods

Animals and surgery

Male Wistar rats weighing 200–250 g were used throughout the experiments. They were housed in a temperature-controlled environment under a light (0700–1900 hours)/dark (1900–0700 hours) cycle with free access to food and water. Behavioural testing was performed between 1000 hours and 1500 hours. For stereotaxic implantation of cannulae, the rats were anaesthetized with sodium pentobarbitone (50 mg/kg IP) and mounted in a stereotactic apparatus (Narishige, Japan). Guide cannulae (0.5 mm o.d., 0.3 mm i.d., 6.0 mm length) were implanted into the nucleus accumbens according to previously described procedures (Prinsep et al. 1994). The coordinates based on the atlas of Paxinos and Watson (1986) were: anterior = 10.6 mm, vertical = 8.0 mm, lateral = 0.5 mm for the shell; and anterior = 10.6 mm, vertical = 7.0 mm, lateral = 1.2 mm for the core. To avoid the ventricular system, the cannulae directed at the shell were angled 21 degrees from the mid-sagittal plane and those directed at the core 18 degrees from that plane. Damage to the target site was minimized by implanting the tips of the guide cannula 1.2 mm (core) or 2.0 mm (shell) above the desired injection site. Wire stylets were placed in the guide cannulae to prevent occlusion. The animals were allowed at least 1 week recovery from the operation. Rats were used only once. All experiments were performed according to institutional and national guideline of animal experimentation.

Intracerebral microinjection and drugs

The drugs used were quinpirole hydrochloride (LY 171555, Research Biochemicals International), a dopamine D2/D3 receptor agonist; L-sulpiride (Ravizza), a dopamine D2 receptor antagonist; SKF 38393 hydrochloride [(±)-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol hydrochloride, Research Biochemicals International], a dopamine D1 receptor agonist; and SCH 23390 [(±)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol hydrochloride, Research Biochemicals International], a dopamine D3 receptor antagonist. Apart from L-sulpiride which was dissolved in a few drops of diluted acetic acid and then diluted with saline (0.9% w/v NaCl solution), all drugs were dissolved in saline. Quinpirole and SKF 38393 were given as a cocktail, where appropriate. All solutions were prepared immediately before use. For a unilateral intracerebral microinjection, the rat was held manually while the stylet was removed and the injection needle (31 gauge) was lowered through the guide cannula until it protruded 1.2 mm (core) or 2.0 mm (shell) beyond the tip. The needle was connected to a Hamilton syringe and the injection was given slowly in a volume of 0.5 μl over 30 s, after which the needle was left in place for a further 30 s. When dopamine receptor antagonists were given into the nucleus accumbens, they were administered 10 min before the injection of the agonist(s). The doses of the dopamine receptor antagonists used have been found to be highly effective in our previous studies on contralateral turning (Saigusa et al. 1995).

Behavioural methods

The rats were placed individually in a circular Perspex chamber (60 cm diameter and 30 cm high) 1 h before the start of the experiment. To allow detailed observation of the turning behaviour, the limb stepping patterns and the type of spinal curvature, a mirror was mounted underneath the chamber at an angle of 30° and the image was recorded on videotape for off-line analysis. Contralateral and ipsilateral turnings (defined as complete 360° turns) were counted visually by a trained observer who had no prior knowledge of the drug treatment. Previously, it has been found that intracerebral administration of L-sulpiride and SCH 23390 can produce dragging of the hindlimbs and a reduced ability to move the head independently of the torso, respectively (Saigusa et al. 1995). Accordingly, particular attention was paid to the display of these phenomena. The behaviour was analyzed during consecutive 5-min periods for 120 min, immediately starting after the injection.

Histology

At the end of each experiment, the rats were deeply anaesthetized with sodium pentobarbitone and perfused transcardially with 10% formalin. The brains were removed, sectioned (50 μm) and stained with cresyl violet to visualize the injection sites.

Data analysis

All values are expressed as means ± SEM and analyzed using either one-way analysis of variance (ANOVA) or two-way ANOVA (group × time) followed by a post hoc Newman-Keuls test, where appropriate. Differences were considered significant when P < 0.05.

Results

Figure 1 gives a survey of the core and shell region in which the injection sites were located; data of rats with injection sites outside the desired region were discarded in the analysis (n = 16). Figure 1 (left side) provides all injection sites in the shell (upper part) and in the core (lower part). Figure 1 (right side) provides a representative example of an actual injection site in the shell (upper part) and in the core (lower part).

Effects of combined injection of quinpirole and SKF 38393 into the core and the shell of the nucleus accumbens

Unilateral injection of the mixture of quinpirole (10 μg) and SKF 38393 (5 μg) into the shell of the nucleus accumbens induced contralateral turning, namely turning directed away from the injection side in all tested rats (n = 6). Ipsilateral turning rarely occurred (data not shown). When given alone in the same dose, quinpirole (n = 6) and SKF 38393 (n = 6) remained ineffective following injections into the shell: the number of turnings in the quinpirole-treated and SKF 38393-treated rats, respectively, did not significantly differ from that found in solvent-treated rats (n = 6). Comparing the effects of the combined treatment with the effects of each single drug or the solvent resulted in a highly significant difference (overall F3,20 = 26.48,
Fig. 1 Left side: all injection sites found in the shell (upper part) and the core (lower part) of the nucleus accumbens. Planes are modified to a series of two or three sections for each brain area from the atlas of Paxinos and Watson (1986); approximate coordinates indicated are in mm anterior to the interaural line. Right side: representative photomicrographs showing drug injection sites within the shell (upper part) and the core (lower part) of the nucleus accumbens.

In contrast, the combined administration of quinpirole and SKF 38393 (n = 10) was far less effective when injected unilaterally into the core (F1,432 = 46.76, P < 0.0001; two-way ANOVA), although the effects were significantly greater than those found after the administration of each drug alone or the solvent (overall F3,24 = 3.80, P < 0.05; Newman-Keuls, P < 0.05 versus SKF 38393 (n = 6), quinpirole (n = 6) or solvent (n = 6), respectively; Fig. 2 lower part).

Analysis of the stepping pattern induced by unilateral injections of the quinpirole-SKF 38393 mixture into the shell showed that the rats displayed the characteristic turning pattern that has been previously described (Saigusa et al. 1995). While pivoting around the contralateral hindlimb, the rats made very tight contralateral turnings. Apart from the occurrence of a small number of forelimb crossing steps, characterized by the sequential occurrence of a closing and an open step (Cools and Jongen-Rêlo 1991), the rats displayed normal forelimb doublets, characterized by the sequential occurrence of a closing and an open step (Cools and Jongen-Rêlo 1991). In contrast, their hindlimb stepping was completely disturbed. Although no hindlimb doublets, characterized by the sequential occurrence of a closing and an open step (Cools and Jongen-Rêlo 1991) were seen, the ipsilateral hindlimb was dragged, while the animal was pivoting contralaterally around its contralateral hindlimb. Often, the contralateral hindlimb stepped backwards. The overall result was a severe head-to-tail curvature during turning and/or sitting.

Effects of L-sulpiride and SCH 23390 on turning elicited by the combined administration of quinpirole and SKF 38393 into the nucleus accumbens

When given into the shell of the nucleus accumbens, both L-sulpiride (25 ng; n = 6) and SCH 23390 (0.5 μg; n = 7) significantly suppressed (P < 0.01, respectively, Newman-Keuls) the contralateral turning induced by unilateral injections of the quinpirole-SKF 38393 mixture into the shell, although the effects of SCH 23390 lasted until 60 min after injection (Fig. 3) due to the short effect of the compound (Koshikawa et al. 1989). Analysis of the resulting stepping pattern revealed the following. First, the characteristic features of the stepping pattern elicited by the cocktail injection, namely dragging of the ipsilateral hindlimb and pivoting...
around the contralateral hindlimb, were fully suppressed by both \( l \)-sulpiride and SCH 23390 in all tested rats. Second, the \( l \)-sulpiride treatment did not produce any additional deficiency in the morphology of the hindlimb stepping. And, third, the SCH 23390 treatment did not affect the rat’s ability to move its head independently of its torso.

**Discussion**

The present study investigated to what extent the contralateral turning known to be elicited by unilateral stimulation of both the dopamine D\(_2\) receptors and the dopamine D\(_1\) receptors in the nucleus accumbens of rats (Saigusa et al. 1993, 1995) is due to stimulation of these receptors in the shell and/or the core of this nucleus. The results show that the combined administration of quinpirole and SKF 38393 was highly effective when injected into the shell of the nucleus accumbens, but far less effective when injected into the core of this nucleus. The nature of the turning did not differ from that previously observed and described by Saigusa et al. (1995). We ascribe the effectiveness of the core injections to leakage of the drugs to the shell; \( l \)-sulpiride which does not diffuse at all and has been found to be an extremely valid tool to differentiate areas in the nucleus accumbens, even within the shell (Cools et al. 1995), fully suppressed the effects of the quinpirole-SKF 39383 mixture, when injected into the shell (see below). Accordingly, we conclude that the shell, but not the core, of the nucleus accumbens is critical for the contralateral turning elicited by the quinpirole-SKF 39383 mixture. As a final remark in this context, it remains to be investigated whether the contralateral turning in this study is (dis)similar to the contralateral turning seen in classic rotation studies on the neostriatum: to our knowledge, there are no studies in which attention has been paid to the individual limb-movements comprising the circling response in that case.

It is well known that quinpirole is a dopamine receptor agonist that has a high affinity not only for dopamine D\(_2\) receptors (Stoof and Kebabian 1981), but also for dopamine D\(_3\) receptors (Sokoloff et al. 1990). Therefore we analyzed the ability of the highly selective dopamine D\(_2\) receptor antagonist \( l \)-sulpiride (Spano et al. 1979) to inhibit the turning behaviour under study. The chosen dose of \( l \)-sulpiride significantly attenuated the turning behaviour and fully suppressed the ipsilateral dragging of the hindlimb as well as the pivoting around the contralateral hindlimb, namely phenomena elicited by the quinpirole-SKF 38393 cocktail. Accordingly, it is concluded that dopamine D\(_2\) receptors are involved in the turning under study. However, it has to be noted that this does not exclude a role of dopamine D\(_3\) receptors. Further studies are necessary to establish their role in this respect. Previously, it has been found that the dose of \( l \)-sulpiride...
which was used in the present study produces deficient hindlimb stepping when it is given to rats receiving an additional injection of the cholinergic agonist carbachol into the same side of the nucleus accumbens (Saigusa et al. 1995). As mentioned, this phenomenon was not seen in the present study, therefore excluding the possible presence of a behavioural rather than a pharmacological inhibition. The occurrence of these unwanted effects in the latter study may imply that activation of cholinoceptive receptors has decreased the dopaminergic activity: for, higher doses of l-sulpiride (50 ng) did produce deficient hindlimb stepping in rats treated with the quinpirole-SKF 38398 cocktail (unpublished data).

The dopamine D₁ antagonist SCH 23390 also strongly attenuated the turning behaviour and fully suppressed the dragging of the ipsilateral hindlimb and the pivoting around the contralateral hindlimb. These findings clearly show that dopamine D₁ receptors were also involved. Previously, it has been found that the dose of SCH 23390 which was used in the present study reduces the rat's ability to move its head independently of its torso when it is given to rats receiving an additional injection of carbachol into the same side of the nucleus accumbens (Saigusa et al. 1995). As mentioned, this was not seen in the present study, therefore excluding again the possible presence of a behavioural inhibition. The occurrence of these unwanted effects in the latter study may also be ascribed to the possibility that carbachol had decreased the dopaminergic activity. However, future studies are required to prove this hypothesis.

The present data show that differences in dopamine neurotransmission between the shell and the core of the nucleus accumbens which are mentioned in the Introduction, have direct consequences for the function of dopamine in these two domains: dopamine D₂ and D₁ receptors in the shell, but not in the core, are involved in contralateral turning elicited by unilateral stimulation of these receptors. Recently, it has been found that this type of turning when elicited from the nucleus accumbens is inhibited by blockade of dopamine receptors in the ventrolateral striatum (Saigusa et al. 1993, 1995). Moreover, the accumbens stimulation also enhances dopamine release in that part of the striatum (Koshikawa et al. 1996). These data strongly suggest that the turning elicited from the shell is somehow funnelled via the ventrolateral striatum. Given these findings, the accumbens-nigro-striatal pathway may at least serve as part of the anatomical substrate of the turning under study. Nevertheless, other projections such as those terminating in the ventral tegmental area, ventral pallidum or mesencephalic locomotor region cannot be excluded yet (Groenewegen et al. 1991; Heimer et al. 1991; Berendse et al. 1992; Wouterlood et al. 1992; Zahm and Heimer 1993). It remains to be investigated whether this also holds true for the oral movements which can be elicited by stimulation of dopamine D₂ and D₁ receptors in the shell, although there are already indications that these movements are funnelled via the projection to the ventral pallidum (Cools et al. 1993, 1995; Prinssen et al. 1994).

In summary, the present study provides evidence that the dopamine D₂ and D₁ receptors in the shell, but not the core, of the nucleus accumbens play a critical role in the contralateral turning elicited by unilateral stimulation of these receptors in this nucleus.

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