

Dissimilarities between cholinergic and dopaminergic turning elicited by nucleus accumbens stimulation in freely moving rats

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Abstract

Contralateral turning was produced by unilateral injection of carbachol (0.5, 2.5, 5 μ g) into the nucleus accumbens, but not into the dorsal or ventral striatum. This behaviour was inhibited by muscarinic M₁ acetylcholine receptor blockade in the nucleus accumbens, and less effectively by blockade of muscarinic M₂ and nicotinic acetylcholine receptors. Unilateral injection of a mixture of the dopamine D₁ receptor agonist 1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol (SKF 38393, 5 μ g) and the dopamine D₂ receptor agonist quinpirole (10 μ g) also produced contralateral turning. The stepping pattern, however, completely differed from that induced by carbachol. The number of carbachol-induced turnings was reduced by dopamine D₁ or D₂ receptor blockade (8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol (SCH 23390) and *l*-sulpiride, respectively) in the nucleus accumbens. However, the reduction was due to a change in the turning pattern. Blockade of muscarinic acetylcholine receptors in the nucleus accumbens did not change the contralateral turning induced by unilateral injection of dopamine receptor agonists into the nucleus accumbens. The results demonstrate that there is no functional interaction between the cholinergic and dopaminergic substrates involved, although blockade of the dopamine receptors elicited behavioural deficits that competed with the turning elicited by carbachol. The contralateral turning elicited by carbachol injection into the nucleus accumbens requires an intact dopamine activity at the level of dopamine D₁ and D₂ receptors in the ipsilateral, but not contralateral, ventrolateral striatum.

Keywords: Turning behavior; Dopaminergic/cholinergic receptor interaction; Nucleus accumbens; Ventrolateral striatum; Carbachol; Turning pattern

1. Introduction

Unilateral manipulation of dopaminergic activity in particular brain regions is known to elicit unilateral turning in freely moving rats. This turning is believed to involve two processes, viz. the process of postural asymmetry, which keeps the animal curved in one direction (postural component), and the process of locomotion (locomotion component), which is the drive for active circling in the direction of the postural asymmetry (Pycck and Marsden, 1978; Pycck, 1980). The process of postural asymmetry has been ascribed to the striatum, whereas the process of locomotion has

been ascribed to the nucleus accumbens (Kelly and Moore, 1976; Moore and Kelly, 1977; Ziegler and Szechtman, 1988,1990). This so-called two-component hypothesis is based upon studies with unilaterally lesioned rats. With unilateral injections of dopaminergic agents into dopaminergic terminal areas, however, it has been found that only unilateral injections into the nucleus accumbens, but not into the dorsal or ventral striatum, elicit contralateral turning (Colle and Wise, 1991; Messier et al., 1991a,b; Saigusa et al., 1993). Still, the ventral striatum does play a role in this effect, since blockade of dopamine D₁/D₂ receptors in the ipsilateral ventral striatum completely abolishes the accumbens-elicited contralateral turning (Saigusa et al., 1993). Since unilateral stimulation of cholinergic receptors in the nucleus accumbens also produces contralateral

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turning (McKenzie et al., 1991), the question arose to what extent this cholinergic contralateral turning is related to the dopaminergic contralateral turning elicited by stimulation of the nucleus accumbens. For that reason, the following experiments were performed. First, the effects of unilateral injections of carbachol into the nucleus accumbens, dorsal and ventral striatum were analysed in freely moving rats. Given the fact that the stepping pattern occurring during unilateral turning varies according to the treatment chosen (Ziegler and Szechtman, 1988; Cools et al., 1989; Cools and Jongen-Relo, 1991; Saigusa et al., 1993; Koshikawa, 1994), the stepping pattern was analysed. Next, a dose-effect curve was made, and the nature of the acetylcholine receptors involved was studied by using nicotinic, muscarinic M_1 and muscarinic M_2 receptor antagonists. Secondly, it was investigated to what extent the contralateral turning elicited by unilateral stimulation of acetylcholine receptors in the nucleus accumbens is influenced by dopaminergic agents given into the nucleus accumbens, and to what extent the contralateral turning elicited by unilateral stimulation of dopamine D_1 and D_2 receptors in the nucleus accumbens is influenced by cholinergic agents given into the nucleus accumbens. Finally, it was investigated whether the contralateral turning elicited by unilateral stimulation of acetylcholine receptors in the nucleus accumbens is blocked by inhibition of dopamine D_1 and D_2 receptors in the ipsilateral ventral striatum. As mentioned, such a ventral striatum manipulation completely abolishes the contralateral turning elicited by unilateral stimulation of dopamine receptors in the nucleus accumbens (Saigusa et al., 1993).

2. Materials and methods

2.1. Animals and surgery

Male Wistar rats weighing 200–250 g were used throughout the experiments. They were housed in a temperature-controlled environment and under a light (07:00–19:00 h)/dark (19:00–07:00 h) cycle with free access to food and water. Behavioural testing was performed between 10:00 h and 15:00 h. For stereotactic implantation of cannulas, the rats were anaesthetized with sodium pentobarbitone (50 mg/kg i.p.) and mounted in a stereotactic apparatus (Narishige, Japan). Guide cannulas (0.5 mm o.d., 0.3 mm i.d.) were implanted into the dorsal (ant. 8.6–9.5, vert. 6.0–6.5, lat. 3.0–3.5) or ventrolateral (ant. 8.0–8.8, vert. 3.0–3.5, lat. 3.5–4.0) parts of the striatum and/or the nucleus accumbens (ant. 10.2–10.7, vert. 3.0–3.5, lat. 1.5–2.0) according to the atlas of Paxinos and Watson (1986) and secured to the skull with stainless screws and

dental acrylic cement. The nucleus accumbens cannulas were angled 20° from the mid-sagittal plane to avoid the ventricular system. Damage to the target site was minimized by implanting the tips of the guide cannulas 1.2 mm above the desired injection site. Wire stylets were placed in the guide cannulas to prevent occlusion. The animals were allowed at least 1 week post-operative recovery before behavioural testing and were used only once.

2.2. Intracerebral microinjection and drugs

The drugs used were carbachol (Sigma), a non-selective acetylcholine receptor agonist; methylscopolamine (Sigma), a non-selective muscarinic acetylcholine receptor antagonist; hexamethonium bromide (Sigma), a nicotinic acetylcholine receptor antagonist; mecamylamine hydrochloride (Sigma), a nicotinic acetylcholine receptor antagonist; pirenzepine dihydrochloride (Research Biochemicals International), a selective muscarinic M_1 receptor antagonist; methoctramine hydrochloride (Research Biochemicals International), a selective muscarinic M_2 receptor antagonist; *cis*-(*Z*)-flupentixol dihydrochloride (Lundbeck), a dopamine D_1/D_2 receptor antagonist; SKF 38393 hydrochloride ((±)-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diol hydrochloride, Research Biochemicals International), a dopamine D_1 receptor agonist; quinpirole hydrochloride (LY 171555, Research Biochemicals International), a dopamine D_2/D_3 receptor agonist; SCH 23390 ((±)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol maleate, Schering Co.), a dopamine D_1 receptor antagonist and *l*-sulpiride (Ravizza), a dopamine D_2 receptor antagonist. *l*-Sulpiride was dissolved in a minimal volume of diluted acetic acid and then diluted with saline (0.9% w/v NaCl solution) for injection. Other drugs were dissolved in saline immediately before use. For bilateral intracerebral microinjections, the rats were held manually while the stylets were removed and the injection needles (31 gauge) were lowered through the guide cannulas until they protruded 1.2 mm beyond the tip. The needles were connected to Hamilton syringes and the drugs were given slowly in a volume of 0.5 µl over 30 s to each side, after which the needles were left in place for a further 30 s. The extent of diffusion, estimated from the spread of the same volume of dye (thionin), was less than 1 mm in diameter. When dopamine receptor or acetylcholine receptor antagonists were given into the nucleus accumbens or the ventral striatum, they were administered 10 min before the injection of the other drug. The doses of the dopamine receptor antagonists have previously been found to be highly effective in our studies on contralateral turning induced by dopamine receptor agonists (Saigusa et al., 1993).

2.3. Behavioural methods

The rats were placed individually in a circular chamber (60 cm diameter) with 30-cm high Perspex sides at least 1 h before the start of the experiment. To allow detailed observation of the limb stepping patterns and spinal curvature, a mirror was mounted underneath the chamber at an angle of 30° and the image was recorded on videotape for later 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. Description of the stepping pattern involved analysis of the presence/absence of the following items: (1) lateral head movements, defined as lateral movements of the head in relation to the upper torso; (2) lateral torso movements, defined as lateral movements of the upper torso in relation to the lower torso; (3) lateral pelvic movements, defined as lateral movements of the lower torso in relation to the hindquarters; (4) normal hindlimb stepping during turning, characterized by the sequential occurrence of two forward steps (hindlimb forward steps: Cools and Jongen-Relo, 1991); (5) apomorphine-induced hindlimb stepping, characterized by the sequential occurrence of a closing and an open step (hindlimb doublet: Cools and Jongen-Relo, 1991); (6) normal forelimb stepping during turning, characterized by the sequential occurrence of a closing and an open step (forelimb doublet: Cools and Jongen-Relo, 1991); (7) dexamphetamine-induced forelimb stepping, characterized by the sequential occurrence of a crossing and an open step (forelimb crossing step: Cools and Jongen-Relo, 1991); (8) pivoting, characterized by turning around one leg (Szechtman et al., 1985); (9) uncoupling of lateral movements of the torso and the limbs, causing the legs to lag behind (dragging). Observations were made during consecutive 5-min periods for 90–120 min, starting immediately after the injection.

2.4. Histology

At the end of each experiment, the rats were deeply anaesthetized with sodium pentobarbitone and perfused transcardially with 10% of formalin. The brains were removed, sectioned (50 μ m) and stained with cresyl violet to visualize the injection site (Fig. 1) and only data from animals in which the injections were correctly placed were analysed.

2.5. Data analysis

All values are expressed as means \pm S.E.M. and analysed using either one-way analysis of variance (ANOVA) or two-way ANOVA (group \times time) followed by a post-hoc Newman-Keuls test, where appropriate. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Effects of intracerebral challenge with acetylcholine receptor agonist and antagonists

Unilateral injection of carbachol (0.5, 2.5 and 5 μ g/0.5 μ l) into the nucleus accumbens induced dose-dependent contralateral turnings: the rats made very wide circles, directed away from the injection side. Analysis of the number of contralateral turnings revealed that the effect was dose-dependent (Fig. 2). Ipsilateral turning rarely occurred (data not shown). Methylscopolamine (0.01, 0.1 and 2.5 μ g) and pirenzepine (0.01, 0.1 and 1 μ g) significantly inhibited the effect of carbachol (5 μ g) in a dose-dependent manner (Fig. 3A and B). The same holds true for methocytamine (0.1, 1 and 5 μ g), mecamlamine (2.5, 5 and 10 μ g) and hexamethonium (1, 2.5 and 5 μ g): they all significantly reduced the response to carbachol (5 μ g), although much higher doses were required (Fig. 3C–E). The carbachol-induced stepping pattern (see section 3.2) was not altered by the acetylcholine receptor antagonists.

In contrast to the effect of carbachol injections into the nucleus accumbens, nearly no contralateral turning was seen after such injections into the dorsal or ventral striatum (Fig. 4). Carbachol injections into the ventral striatum, however, produced many jaw movements and profound salivation (data not shown).

3.2. Stepping pattern induced by cholinergic and dopaminergic manipulation of the nucleus accumbens

Analysis of the stepping pattern induced by unilateral carbachol injections into the nucleus accumbens showed that the rats did not show any abnormal stepping turning: they displayed hindlimb forward steps and forelimb doublets, whereas hindlimb doublets, forelimb crossing steps, pivoting and dragging were absent. The sequential occurrence of lateral movements of the head, torso and pelvis seen after carba-

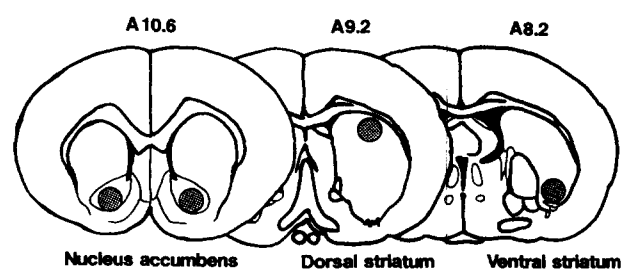


Fig. 1. Injection sites within the nucleus accumbens, the dorsal striatum and the ventral striatum. Planes are modified to a series of 2 or 3 sections for each brain area from the atlas of Paxinos and Watson (1986); approximate coordinates indicated are in mm anterior to the interaural line.

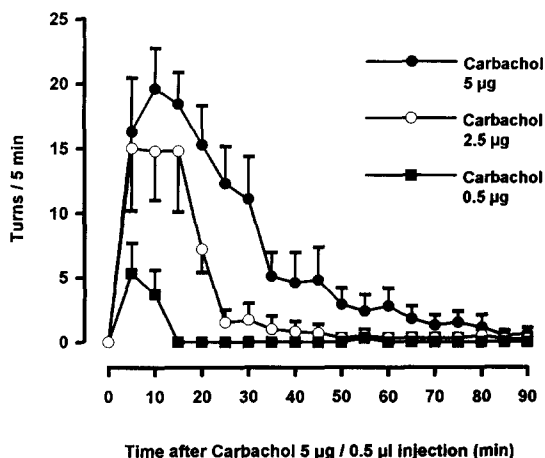


Fig. 2. Effects of unilateral injection into the nucleus accumbens of carbachol 0.5 $\mu\text{g}/0.5 \mu\text{l}$ (■), 2.5 $\mu\text{g}/0.5 \mu\text{l}$ (○) or 5 $\mu\text{g}/0.5 \mu\text{l}$ (●) on production of contraversive turning. The data are expressed as the mean number of turns occurring in 5-min observation periods ($n = 6-8$). Vertical bars indicate S.E.M.

chol did not differ from that seen in solvent-treated rats (Fig. 5A). When compared with solvent-treated rats, the carbachol-treated rats started to increase forward stepping, resulting sometimes in running. While running forward, there was a gradual transition from pure, contralateral head movements to pure, contralateral torso movements and then to pure, contralateral pelvic movements (Fig. 5A). The ultimate result was very wide circles.

Analysis of the stepping pattern induced by unilateral injections of the mixture of SKF 38393 (5 μg) and quinpirole (10 μg) into the nucleus accumbens, namely a treatment known to produce contralateral turning (Saigusa et al., 1993) revealed a completely different effect. 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, the rats displayed normal forelimb doublets. In contrast, their hindlimb stepping was completely disturbed. Although no hindlimb doublets were seen, the ipsilateral hindlimb was dragged, while the animal was pivoting contralaterally around its contralateral hindlimb. Often, the contralateral hindlimb stepped backwards. The sequential occurrence of lateral movements of the head, torso and pelvis seen after the injection did not differ from that seen in the solvent-treated rats. When compared with their controls, the treated rats started to decrease forwards stepping with their hindlimbs, resulting sometimes in the animals sitting immobile. While slowing down forward movements, there was a gradual transition from pure, contralateral head movements to pure, contralateral torso movements and then to pure, contralateral pelvic movements. The overall result was a severe

head-to-tail curvature during turning and/or sitting (Fig. 5B).

3.3. Effects of intra-accumbens injections of SCH 23390 and *l*-sulpiride on carbachol-induced turning

When given into the nucleus accumbens, both SCH 23390 (0.1 and 0.5 μg) and *l*-sulpiride (25 and 50 ng) significantly suppressed the contralateral turning induced by unilateral injections of carbachol (5 μg) into the nucleus accumbens (Fig. 6). However, the effect of SCH 23390 was not dose-dependent. Moreover, the effect of carbachol was not completely blocked. Analy-

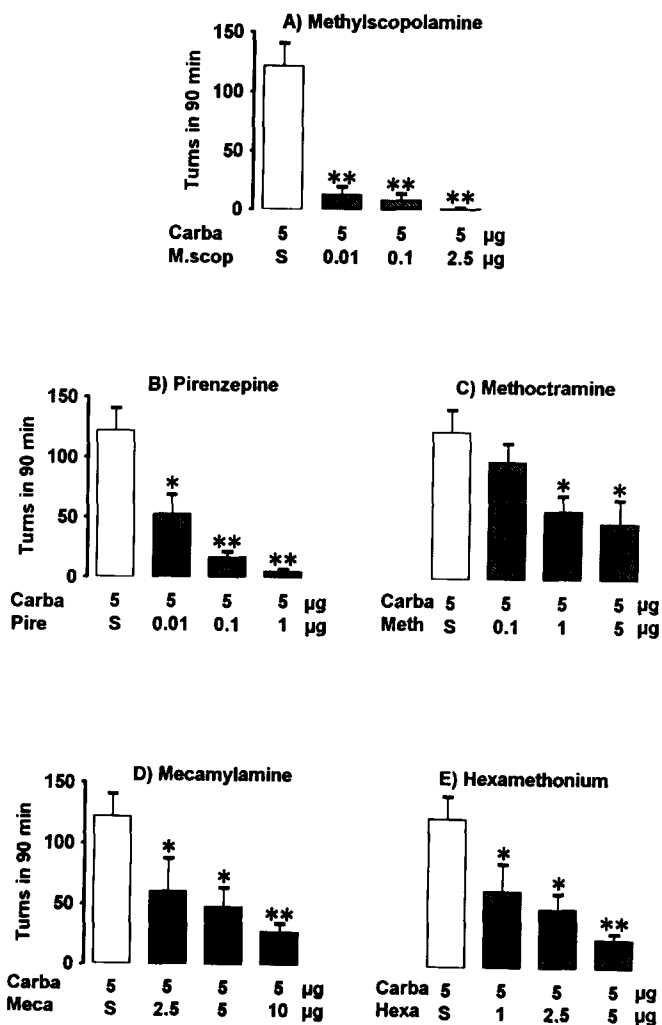


Fig. 3. Contraversive turning induced by unilateral injection of carbachol (5 $\mu\text{g}/0.5 \mu\text{l}$) into the nucleus accumbens of rats given 10 min after injecting saline (open column), methylscopolamine (0.01, 0.1 or 1 $\mu\text{g}/0.5 \mu\text{l}$, A), pirenzepine (0.01, 0.1 or 1 $\mu\text{g}/0.5 \mu\text{l}$, B), methoctramine (0.1, 1 or 5 $\mu\text{g}/0.5 \mu\text{l}$, C), mecamylamine (2.5, 5 or 10 $\mu\text{g}/0.5 \mu\text{l}$, D) or hexamethonium (1, 2.5 or 5 $\mu\text{g}/0.5 \mu\text{l}$, E) into the same side of the nucleus accumbens. The data are expressed as the mean number of total turns occurring in a 90-min observation period ($n = 6-8$). Vertical bars indicate S.E.M. * $P < 0.05$, ** $P < 0.01$ (Newman-Keuls test).

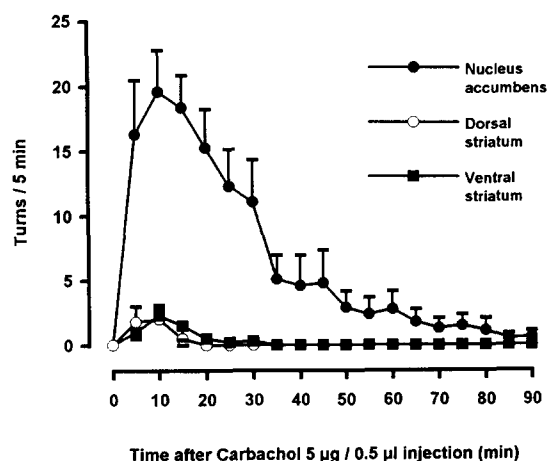


Fig. 4. Effects of unilateral injection of carbachol ($5 \mu\text{g}/0.5 \mu\text{l}$) into the nucleus accumbens (●), the dorsal striatum (○) or the ventral striatum (■) on production of contraversive turning. The data are expressed as the mean number of turns occurring in 5-min observation periods ($n = 6-8$). Vertical bars indicate S.E.M.

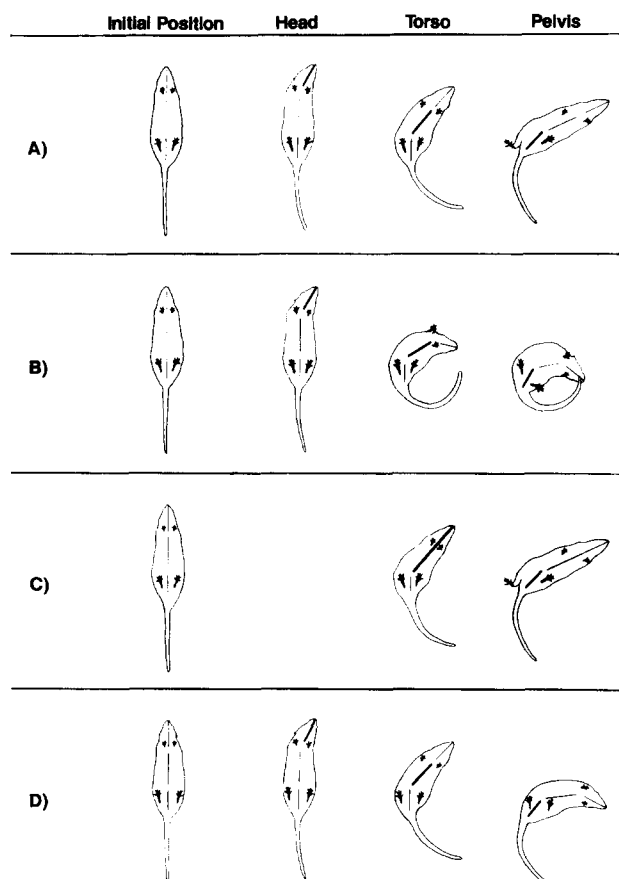


Fig. 5. A schematic illustration showing sequential occurrence of lateral movements of the head, torso and pelvis seen after injection of drugs into the ipsilateral nucleus accumbens. The axis of the moving part of the trunk closest to the stationary base is represented by a thick barline. A: saline or carbachol ($5 \mu\text{g}/0.5 \mu\text{l}$); B: a mixture of SKF 38393 ($5 \mu\text{g}$) and quinpirole ($10 \mu\text{g}$) in $0.5 \mu\text{l}$; C: SCH 23390 ($0.1 \mu\text{g}/0.5 \mu\text{l}$) injected into the nucleus accumbens 10 min before carbachol ($5 \mu\text{g}/0.5 \mu\text{l}$); D: *l*-sulpiride ($25 \text{ ng}/0.5 \mu\text{l}$) given into the nucleus accumbens 10 min before carbachol ($5 \mu\text{g}/0.5 \mu\text{l}$).

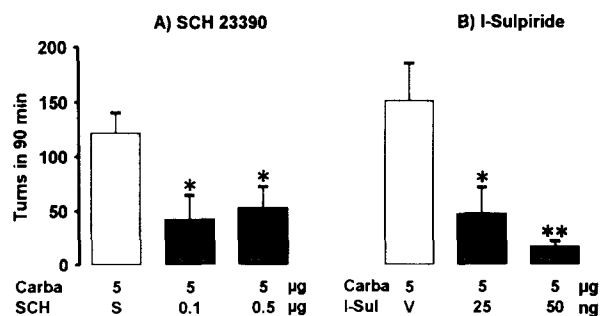


Fig. 6. Contraversive turning induced by unilateral injection of carbachol ($5 \mu\text{g}/0.5 \mu\text{l}$) into the nucleus accumbens of rats given 10 min after injecting saline (open column), SCH 23390 (0.1 or $0.5 \mu\text{g}/0.5 \mu\text{l}$, A) or *l*-sulpiride (25 or $50 \text{ ng}/0.5 \mu\text{l}$, B) into the same side of the nucleus accumbens. The data are expressed as the mean number of total turns occurring in a 90-min observation period ($n = 6-9$). Vertical bars indicate S.E.M. * $P < 0.05$, ** $P < 0.01$ (Newman-Keuls test).

sis of the resulting stepping pattern revealed that the carbachol-induced stepping was differentially affected by SCH 23390 and *l*-sulpiride (Fig. 5C and D).

SCH 23390 did not alter the carbachol-induced stepping of the hindlimbs. Neither did it alter the carbachol-induced increase in forward stepping. In contrast, SCH 23390 affected forelimb stepping: the forelimbs were often simultaneously lifted as soon as the rat turned contralaterally. Apart from these abnormal forelimb steps, normal forelimb doublets were present when the rat touched the wall. Furthermore, SCH 23390 affected the neck joint, preventing the rat from displaying pure contralateral head movements. When the head was moved laterally, the torso simultaneously moved laterally: the head appeared to be fixed to the torso (Fig. 5C). Consequently, the rats had difficulties with executing carbachol-induced turning.

l-Sulpiride affected carbachol-induced turning in a different manner. It disturbed the hindlimb stepping; there was an uncoupling between lateral movements of the torso and those of the hindlimbs, resulting in dragging of these limbs when the head and torso were fully rotated towards the contralateral side (Fig. 5D). Furthermore, the number of hindlimb steps was reduced, decreasing thereby the carbachol-induced increase in forward stepping. Finally, the rats often had splayed hindlimbs. In contrast, *l*-sulpiride did not affect the normal forelimb stepping. Overall, *l*-sulpiride reduced the number of the carbachol-induced contralateral turnings because of deficient hindlimb stepping.

3.4. Effects of intra-accumbens injections of methylscopolamine upon contralateral turning elicited by SKF 38393 and quinpirole from the nucleus accumbens

Intra-accumbens administration of methylscopolamine in a dose that inhibited the carbachol-induced

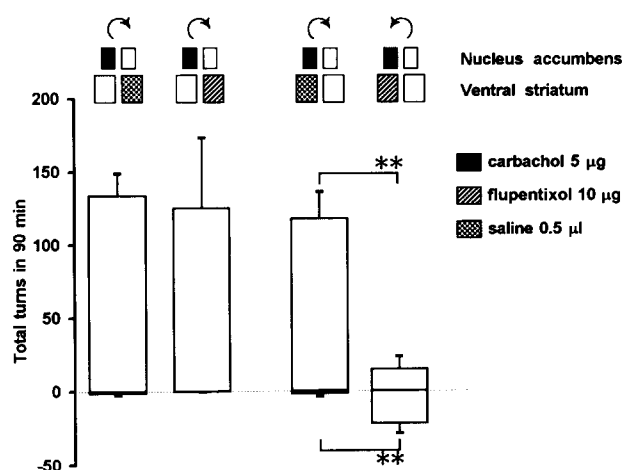


Fig. 7. Turning induced by unilateral injection of carbachol ($5 \mu\text{g}/0.5 \mu\text{l}$) into the nucleus accumbens (filled symbol) of rats given 10 min after injecting saline (stippling) or *cis*-(Z)-flupentixol (hatched) into the ipsilateral or contralateral ventral striatum as indicated in the upper portion of the figure. The data are expressed as the mean number of total turns occurring in a 90-min observation period ($n = 6-10$) and the dominant direction of turning is indicated by arrows. Vertical bars indicate S.E.M. ** $P < 0.01$ compared to appropriate saline control (Newman-Keuls test).

turning (section 3.1: $2.5 \mu\text{g}$) did not affect the contralateral turning elicited by intra-accumbens administration of the mixture of SKF 38393 ($5 \mu\text{g}$) and quinpirole ($10 \mu\text{g}$): control rats showed 228.3 ± 27.0 turnings/120 min ($n = 7$), whereas the methylscopolamine-pretreated rats showed 263.0 ± 128.9 turnings/120 min ($n = 9$).

3.5. Effects of ventral striatal injections of *cis*-(Z)-flupentixol on carbachol-induced turning elicited from the nucleus accumbens

The carbachol-induced turning was significantly suppressed by injection of *cis*-(Z)-flupentixol into the ipsilateral, but not contralateral, ventrolateral striatum (Fig. 7). As long as the rats were still turning to the contralateral side, there was no significant change in the carbachol-induced stepping pattern. *cis*-(Z)-Flupentixol just reduced the carbachol-induced increase in forward stepping and the number of carbachol-induced contralateral head movements. Following the disappearance of contralateral turning, the rats often remained immobile for a short period. Then, some rats ($n = 4$, 40% of tested rats) started again to turn at 5–20 min after the carbachol injection, but now towards the ipsilateral side. Moreover, one rat showed turning exclusively to the ipsilateral side. The resulting stepping pattern during the ipsilateral turning differed completely from the carbachol-induced stepping pattern. In fact, the stepping pattern seen after the combined *cis*-(Z)-flupentixol-carbachol treatment was quite

similar to that seen after injection of the mixture of SKF 38393 ($5 \mu\text{g}$) and quinpirole ($10 \mu\text{g}$) into the nucleus accumbens, apart from the direction (ipsilateral vs. contralateral). The only difference seen was that the *cis*-(Z)-flupentixol-carbachol-treated rats that showed ipsilateral turning at the end of the observation period, also showed deficient forelimb stepping, due to dragging of the contralateral forelimb during the ipsilateral turning.

4. Discussion

The goal of the present study was 2-fold. First, it was investigated to what extent unilateral injections of the non-selective acetylcholine receptor agonist carbachol into the nucleus accumbens, ventral and dorsal striatum of freely moving rats produces turning behaviour that is specific for stimulation of nicotinic, muscarinic M_1 and/or M_2 acetylcholine receptors (cf. McKenzie et al., 1991). Second, it was investigated to what extent contralateral turning elicited by unilateral injection of carbachol into the nucleus accumbens shows similarities and/or dissimilarities with the contralateral turning elicited by unilateral injection of a mixture of dopamine D_1 and D_2 receptor agonists into the nucleus (cf. Saigusa et al., 1993). It is well known that striatal administration of dopamine receptor agonists produces either (a) effects that are fully similar to those elicited by striatal administration of cholinergic agonists or (b) effects that are diametrically opposite to those elicited by striatal administration of cholinergic agonists, depending on the striatal area studied (Bartholini, 1980; Kikuchi de Beltrán et al., 1992; for reviews: Cools, 1977; Scheel-Krüger, 1985).

As shown in section 3.1, unilateral injection of carbachol produced contralateral turning only, when administered into the nucleus accumbens, but not into the ventral or dorsal striatum. The turning was specific and selective for stimulation of acetylcholine receptors. First, the carbachol-induced turning was dose-dependent. Second, it could be antagonized by non-selective muscarinic acetylcholine receptor antagonists as well as by selective muscarinic M_1 , M_2 acetylcholine receptor antagonists and by selective nicotinic acetylcholine receptor antagonists (Fig. 3). Since pirenzepine, a selective muscarinic M_1 acetylcholine receptor antagonist, was the most potent inhibitor, it appears that especially the muscarinic M_1 acetylcholine receptor subtype in the nucleus accumbens was involved in the carbachol-induced turning, although a role for the other subtypes cannot be dismissed. Third, the stepping pattern induced by carbachol differed completely from that elicited by the mixture of the dopamine D_1 receptor agonist SKF 38393 and the dopamine D_2 receptor agonist quinpirole (see section 3.2). Thus, it is con-

cluded that the muscarinic M_1 acetylcholine receptors in the nucleus accumbens play a crucial role in the display of the contralateral turning elicited by unilateral administration of carbachol into the nucleus accumbens. However, a titration study, using various mixtures of selective muscarinic M_1 , M_2 and nicotinic acetylcholine receptor agonists, is required to establish the distinct contribution of each receptor subtype.

As mentioned, the stepping pattern seen during the contralateral turning elicited by carbachol injection into the nucleus accumbens completely differed from that elicited by unilateral administration of the mixture of the dopamine D_1 receptor agonist SKF 38393 and the dopamine D_2 receptor agonist quinpirole into the nucleus accumbens: the stepping pattern elicited by carbachol did not differ from that seen in solvent-treated rats, whereas the stepping pattern elicited by the dopamine agonists was marked by abnormal movements, especially of the hindlimbs. These data suggest that two different neurobiological substrates were involved. In fact, the following findings indicate that these substrates are rather loosely coupled to each other. First, the dopamine D_1 receptor antagonist SCH 23390 did not antagonize the carbachol-induced contralateral turning in a pharmacological manner. Apart from the findings that the effect of carbachol could be neither dose-dependently nor fully suppressed by SCH 23390, the attenuation of the carbachol-induced contralateral turning was due to the occurrence of additional deficits in moving the forelimbs and/or the neck joint, providing evidence that a behavioural instead of a pharmacological inhibition was responsible for the reduction in the number of carbachol-induced turnings. Second, as the dopamine D_2 receptor antagonist sulpiride did not antagonize the carbachol-induced turning in a pharmacological manner, the attenuation of carbachol-induced contralateral turning was due to additional deficits in moving the hindlimbs, providing evidence that a behavioural instead of a pharmacological inhibition was responsible for the reduction in the number of carbachol-induced turnings. In this context it is relevant to note that earlier published studies have shown that inhibition of dopamine receptors in the nucleus accumbens of naive or dexamphetamine-treated rats does indeed produce deficits in the stepping pattern (Cools and Jongen-Relo, 1991; Cools, 1992). Third, the non-selective muscarinic acetylcholine receptor antagonist methylscopolamine was unable to alter the contralateral turning elicited by the unilateral administration of a mixture of the dopamine D_1 and dopamine D_2 receptor agonists into the nucleus accumbens. On the basis of these data we conclude that the nucleus accumbens contains at least three distinct neurobiological substrates, each of them having its own particular function in directing certain behaviours and/or body parts and, accordingly, having

its own particular output pathways (cf. Cools, 1992): a cholinergic substrate subserving the normal recruitment of pure lateral head movements, pure lateral torso movements and pure lateral pelvic movements, respectively; a dopamine D_1 receptor-sensitive substrate subserving the forelimbs and/or the neck joint; and a dopamine D_2 receptor-sensitive substrate subserving the hindlimbs and/or the lower torso. Indeed, it is known that striatal structures contain somatotopically organized modules marked by their own input and output pathways (DeLong et al., 1992). The data suggest that these modules are differentially regulated by acetylcholine, dopamine D_1 and D_2 receptors. In this respect the nucleus accumbens does not differ from the striatum, of which it is known that dopamine D_1 and dopamine D_2 receptors are predominantly located on striato-nigral and striato-pallidal output pathways, respectively (Gerfen, 1992). Combined anatomical-biochemical studies are necessary to provide direct evidence in favour of this hypothesis.

The finding that carbachol was effective only after administration into the nucleus accumbens, but not when administered into the dorsal or ventral striatum, underscores our previously reported notion that stimulation of particular receptors in the nucleus accumbens is sufficient for producing turning behaviour (Saigusa et al., 1993; Koshikawa, 1994; cf. Colle and Wise, 1991; McKenzie et al., 1991; Messier et al., 1991a,b). Previously, we have hypothesized that stimulation of particular receptors in the nucleus accumbens is sufficient for activating both the process of locomotion (postulated to be mediated by the nucleus accumbens: see Introduction) and the process of postural asymmetry (postulated to be mediated by the striatum: see Introduction), since such a stimulation alters not only the neurotransmission activity in the nucleus accumbens, but also the neurotransmission activity in the ventral striatum (Saigusa et al., 1993; Koshikawa, 1994). Apart from our ongoing microdialysis studies that provide direct evidence in favour of our hypothesis, this hypothesis is supported by the present finding that inhibition of dopamine D_1 and D_2 receptors in the ipsilateral ventrolateral striatum abolished the turning elicited by unilateral injection of carbachol into the nucleus accumbens. It is unlikely that the inhibition in question was due to behavioural inhibition, since the reduction in carbachol-induced contralateral turning preceded the changes seen in the stepping pattern during the ipsilateral turning that appeared after the disappearance of the carbachol-induced contralateral turning. To what extent such changes may have contributed to our earlier reported observation that inhibition of dopamine D_1 and D_2 receptors in the ipsilateral ventrolateral striatum suppresses the contralateral turning elicited by unilateral administration of the dopamine D_1 and D_2 receptor agonists into the nucleus accumbens

bens is not clear, since the stepping pattern was not analysed at that time (Saigusa et al., 1993).

In conclusion, the present study shows that the contralateral turning elicited by unilateral administration of carbachol into the nucleus accumbens requires stimulation of muscarinic M_1 acetylcholine receptors in the nucleus accumbens and, to a less degree, stimulation of muscarinic M_2 and nicotinic acetylcholine receptors. The present study also shows that this carbachol-induced contralateral turning differs from the contralateral turning elicited by unilateral administration of the mixture of the dopamine D_1 receptor agonist SKF 38393 and the dopamine D_2 receptor agonist quinpirole into the nucleus accumbens. Moreover, the present study shows that there is no functional interaction between the cholinergic and dopaminergic substrates involved, although blockade of the dopaminergic receptors can elicit behavioural deficits that compete with the behavioural effects of cholinergic stimulation. Finally, the contralateral turning elicited by unilateral stimulation of cholinergic receptors in the nucleus accumbens was found to require intact dopaminergic activity at the level of dopamine D_1 and dopamine D_2 receptors in the ipsilateral ventrolateral striatum for its expression, showing that changing the neurotransmission activity in the nucleus accumbens has direct consequences for the neurotransmission activity in the ipsilateral ventral striatum.

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References

- Bartholini, G., 1980, Interaction of striatal dopaminergic, cholinergic and GABA-ergic neurons: relation to extrapyramidal function, *Trends Pharmacol. Sci.* (Jan.), 138.
- Colle, L.M. and R.A. Wise, 1991, Circling induced by intra-accumbens amphetamine injections, *Psychopharmacology* 105, 157.
- Cools, A.R., 1977, Basic considerations on the role of concertedly working dopaminergic, GABA-ergic, cholinergic and serotonergic mechanisms within the neostriatum and nucleus accumbens in locomotor activity, stereotyped gnawing, turning and dyskinetic activities, in: *Cocaine and Other Stimulants*, eds. E.H. Ellinwood and M.M. Kilbey (Plenum Press, New York), *Adv. Behav. Biol.* Vol. 21, p. 97.
- Cools, A.R., 1992, Striatal structures, dopamine and the mobility gradient model, *Behav. Brain Sci.* 15, 271.
- Cools, A.R. and A.L. Jongen-Relo, 1991, Role of neostriatum and nucleus accumbens in stepping induced by apomorphine and dexamphetamine, *Brain Res. Bull.* 26, 909.
- Cools, A.R., W. Scheenen, D. Eilam and I. Golani, 1989, Evidence that apomorphine and (+)-amphetamine produce different types of circling in rats, *Behav. Brain Res.* 34, 111.
- DeLong, M.R., G.E. Alexander, W.C. Miller and M.D. Crutcher, 1992, Anatomical and functional aspects of basal ganglia-thalamocortical circuits, in: *Function and Dysfunction in the Basal Ganglia*, eds. A.J. Franks, J.W. Ironside and R.H.S. Mindham (Pergamon Press, Oxford) p. 5.
- Gerfen, C.R., 1992, The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia, *Annu. Rev. Neurosci.* 15, 285.
- Kelly, P.H. and K.E. Moore, 1976, Mesolimbic dopaminergic neurons in the rotational model of nigrostriatal function, *Nature* 263, 695.
- Kikuchi de Beltrán, K., N. Koshikawa, T. Saigusa, K. Watanabe, Y. Koshida and M. Kobayashi, 1992, Cholinergic/dopaminergic interaction in the rat striatum assessed from drug-induced repetitive oral movements, *Eur. J. Pharmacol.* 214, 181.
- Koshikawa, N., 1994, Role of the nucleus accumbens and the striatum in the production of turning behaviour in intact rats, *Rev. Neurosci.* (in press).
- McKenzie, J.S., A.D. Shafon and C.A. Stewart, 1991, Intrastriatal dopaminergic agents, muscarinic stimulation, and GABA antagonism compared for rotation responses in rats, *Behav. Brain Res.* 45, 163.
- Messier, C., O. Mrabet and C. Destrade, 1991a, Locomotor bias produced by intra-accumbens injection of dopamine agonists and antagonists, *Pharmacol. Biochem. Behav.* 41, 177.
- Messier, C., O. Mrabet, T.P. Durkin and C. Destrade, 1991b, Bidirectional potentiation between D_1 and D_2 dopamine agonists: effects of unilateral intra-accumbens injections on locomotor activity in mice, *Life Sci.* 49, 43.
- Moore, K.E. and P.H. Kelly, 1977, Biochemical pharmacology of mesolimbic and mesocortical dopaminergic neurons, in: *Psychopharmacology, A Generation of Progress*, eds. M.A. Lipton, A. Di Mascio and K.F. Killiam (Raven Press, New York) p. 126.
- Paxinos, G. and C. Watson, 1986, *The Rat Brain in Stereotaxic Coordinates* (Academic Press, New York, London).
- Pycoc, C.J., 1980, Turning behaviour in animals, *Neuroscience* 5, 461.
- Pycoc, C.J. and C.D. Marsden, 1978, The rotating rodent: a two component system?, *Eur. J. Pharmacol.* 47, 167.
- Saigusa, T., N. Koshikawa, M. Kitamura and M. Kobayashi, 1993, Reevaluation of the two-component hypothesis for turning behaviour by manipulating activities in the striatum and the nucleus accumbens of intact rats, *Eur. J. Pharmacol.* 237, 161.
- Scheel-Krüger, J., 1985, New aspects on the functional role of acetylcholine in the basal ganglia: interactions with other neurotransmitters, in: *Central Cholinergic Mechanisms and Adaptive Dysfunctions*, eds. M.M. Singh, H. Lal and H. Wasburton (Plenum Press, New York) p. 105.
- Szechtman, H., K. Ornstein, P. Teitelbaum and I. Golani, 1985, The morphogenesis of stereotyped behavior induced by the dopamine receptor agonist apomorphine in the laboratory rat, *Neuroscience* 14, 783.
- Ziegler, M. and H. Szechtman, 1988, Differences in the behavioral profile of circling under amphetamine and apomorphine in rats with unilateral lesions of the substantia nigra, *Behav. Neurosci.* 102, 276.
- Ziegler, M. and H. Szechtman, 1990, Relation between motor asymmetry and direction of rotational behaviour under amphetamine and apomorphine in rat with unilateral degeneration of the nigrostriatal dopamine system, *Behav. Brain Res.* 39, 123.