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Behavioural effects of 7-OH-DPAT are solely due to stimulation of dopamine D_2 receptors in the shell of the nucleus accumbens; jaw movements

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Abstract

The goal of this study was to determine whether the dopamine D_3 receptor in limbic structures plays a role in the shell-specific and dopamine-dependent display of jaw movements in rats. When combined with the dopamine D_1 receptor agonist (±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SKF 82958, 5 μg), the putative dopamine D_3 receptor agonist (±)-7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT, 10 μg) produced repetitive jaw movements following injection into the shell, but not the core, of the nucleus accumbens. This behaviour was only partially inhibited by local blockade of dopamine D_1 receptors (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine, SCH 23390, 500 ng), dopamine D_2 receptors (domperidone, 50 and 100 ng) or dopamine $D_{2/3}$ receptors (*l*-sulpiride, 25 ng). Combined blockade of both dopamine D_1 and D_2 receptors in the shell completely antagonized the jaw movements elicited by the cocktail of SKF 82958 and 7-OH-DPAT. Replacing 7-OH-DPAT by another putative dopamine D_3 receptor agonist, *S*(+)-(4*aR*,10*bR*)-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano[4,3-*b*]-1,4-oxazin-9-ol (PD 128,907, 10 μg), in the cocktail did not produce jaw movements, when administered into the shell. Injection of the cocktail of SKF 82958 and 7-OH-DPAT into the ventrolateral striatum, which contains nearly no dopamine D_3 receptors, also elicited jaw movements. It is concluded that mesolimbic dopamine D_3 receptors play no role in the dopamine-dependent and shell-specific jaw movements: the contribution of 7-OH-DPAT in the cocktail of SKF 82958 and 7-OH-DPAT to the display of jaw movements is solely due to its ability to activate dopamine D_2 receptors.

Keywords: Accumbal shell; Accumbal core; Jaw movement; Dopamine D_1/D_2 receptor interaction; Dopamine D_1/D_3 receptor interaction; (Rat)

1. Introduction

Since the discovery of the dopamine D_3 receptor, it has been speculated that these receptors play a role in cognition, emotion and motivated behaviour (Sokoloff et al., 1990; Diaz et al., 1995). This dopamine D_3 receptor is abundantly concentrated in limbic structures, such as the nucleus accumbens and the olfactory tubercle, but nearly absent in striatal structures, such as the ventrolateral striatum (Sokoloff et al., 1990). Recently, evidence has been presented that a subpopulation of dopamine D_3 receptors is located on neurons which serve as a major output route of the shell, but not the core, of the nucleus accumbens (Diaz et al., 1995).

Studies on the function of dopamine D_3 receptors are limited by the lack of selective agonists and antagonists. Nevertheless, there are some agents which appear to have a higher affinity at the dopamine D_3 receptor than at any other dopamine receptor: (±)-7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) is such a compound (Bouthenet et al., 1991; Lévesque et al., 1992). 7-OH-DPAT has been found to act as a selective agonist in a variety of biochemical, electrophysiological and behavioural studies (biochemical studies: Rivet et al., 1994; Sokoloff et al., 1990; electrophysiological studies: Kreiss et al., 1995; behavioural studies: Daly and Waddington, 1993; Damsma et al., 1993; Starr and Starr, 1995; Svens-

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son et al., 1994; Waters et al., 1993). Still, there is also evidence that 7-OH-DPAT may be less selective than originally claimed (biochemical studies: Gonzalez and Sibley, 1995; electrophysiological studies: Freedman et al., 1994; Liu et al., 1994; behavioural studies: Large and Stubbs, 1994). Apart from a few electrophysiological studies in which 7-OH-DPAT has been intracerebrally applied into the substantia nigra, the striatum and the nucleus accumbens (Freedman et al., 1994; Kreiss et al., 1995; Liu et al., 1994), all remaining studies on the function of dopamine D_3 receptors are limited to studies in which 7-OH-DPAT and other putative, selective dopamine D_3 receptor agents are systemically applied. Until now, there is no direct evidence that dopamine D_3 receptors are involved in limbic functions.

Given the abundant presence of dopamine D_3 receptors in the nucleus accumbens of the rat, especially in its shell (Sokoloff et al., 1990; Diaz et al., 1995), the question arises to what extent stimulation of these receptors gives rise to behavioural changes known to be specific for the shell of the nucleus accumbens. Such evidence is required in order to prove that dopamine D_3 receptors play a role in mediating functions of mesolimbic structures.

Recently, it has become evident that the shell plays a critical role in dopamine-specific behaviours, such as oral movements (Cools et al., 1993, 1995; Prinssen et al., 1994) and turning behaviour (Koshikawa et al., 1996a). These studies have shown that administration of a highly specific dose-combination of the dopamine D_1 receptor agonist (±)-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diol (SKF 38393, 5 μg) or (±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SKF 82958, 5 μg) and the dopamine D_2 receptor agonist quinpirole (10 μg) into the shell, but not the core, of the nucleus accumbens is required for the elicitation of jaw movements and turning behaviour in rats, respectively. Since these behaviours are at least partly inhibited by the intra-accumbens administration of either the dopamine D_1 receptor antagonist SCH 23390 or the dopamine D_2 receptor antagonist *l*-sulpiride (Cools et al., 1995; Koshikawa et al., 1996a), it has been suggested that dopamine D_1 and D_2 receptors play a role in the expression of these behaviours. However, a role of dopamine D_3 receptors could not be excluded: quinpirole has a high affinity not only at the dopamine D_2 receptor (Stoof and Kebabian, 1981), but also at the dopamine D_3 receptor (Sokoloff et al., 1990), and *l*-sulpiride has only a marginal lower affinity at the dopamine D_3 receptor than at the dopamine D_2 receptor (Sokoloff et al., 1990). Accordingly, we decided to investigate the behavioural role of dopamine D_3 receptors in the shell of the nucleus accumbens of rats. For that purpose, we replaced quinpirole in the SKF 82958 – quinpirole or SKF 38393 – quinpirole cocktail by the putative dopamine D_3 receptor agonist 7-OH-DPAT and studied the effects of this treatment upon jaw movements and turning behaviour, respectively. This paper reports the outcome of our study on jaw movements, whereas our study on turning behaviour is reported in the accompanying paper (Koshikawa et al., 1996b).

Given the lack of available dopamine D_3 receptor antagonists at the moment, the following strategy was used to assess the specificity of the effects elicited. First, the effects following administration of the cocktail into the shell were compared with those following administration of this cocktail into the core, namely an area containing far less dopamine D_3 receptors than the shell (see above). Second, the cocktail was administered into the ventrolateral striatum, namely an area being also involved in the expression of oral behaviour (Bordi et al., 1989; Kelley et al., 1988; Koshikawa et al., 1989; Delfs and Kelley, 1990), but lacking more or less dopamine D_3 receptors (Sokoloff et al., 1990). Third, we investigated to what extent the effects elicited by this cocktail could be partly or completely antagonized by the dopamine D_1 receptor antagonist *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH 23390) or the dopamine D_2 receptor antagonist domperidone. In an additional series of these experiments, *l*-sulpiride was included in order to establish whether benzamides, such as *l*-sulpiride, which inhibit both dopamine D_2 and D_3 receptors, is more effective than dopamine D_2 receptor antagonists, such as domperidone, which primarily inhibit the dopamine D_2 receptor (Sokoloff et al., 1990). Fourth, the effect of a mixture of SCH 23390 and *l*-sulpiride or domperidone upon the effects elicited by the cocktail was analysed. Finally, the effect of the cocktail was compared to that of a cocktail, in which 7-OH-DPAT was replaced by another putative dopamine D_3 receptor agonist, *S*(+)-(4*aR*,10*bR*)-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-[1]-benzopyrano[4,3-*b*]-1,4-oxazin-9-ol (PD 128,907). The binding profile of PD 128,907 for dopamine D_3 receptors relative to dopamine D_2 receptors is reported to be more selective (54-fold) than that of 7-OH-DPAT (7-fold) (Sautel et al., 1995). Given the outcome of the present study, it became necessary to exclude the possible involvement of dopamine D_3 receptors in the oral effects seen in our previous study, using a cocktail of SKF 82958 and quinpirole (Cools et al., 1995). Therefore, the effects of a mixture of the dopamine D_1 receptor antagonist SCH 23390 and the dopamine D_2 receptor antagonist domperidone given prior to the SKF 82958-quinpirole cocktail were also analysed following their administration into the shell.

2. Materials and methods

2.1. Surgical procedures

Male Sprague-Dawley rats weighing 260–330 g were anaesthetized with ketamine HCl (10 mg/kg i.p.), supplemented during surgery with halothane (0.5–4% when ap-

propriate). The surgical and recording procedures were as described previously (Koshikawa et al., 1989, 1990a,b, 1991; Cools et al., 1995). A small light-emitting diode was fixed to the mandible and the animal was placed in a stereotactic frame so that the head was kept in constant relation to a light-sensitive transducer which detected the vertical movements of the diode. The spinal cord was transected at C1 level to confine drug-induced jaw movements to the head region. The jaw movements were recorded on a polygraph for later quantification and were counted automatically with a spike trigger. The registration period lasted 240 min. Guide cannulas (0.5 mm o.d., 0.3 mm i.d., 6.0 mm length) were implanted bilaterally into the brain according to previously described procedures (Koshikawa et al., 1989; Prinssen et al., 1994; Cools et al., 1995). The coordinates based on the atlas of Paxinos and Watson (1986) were: anterior = 10.6 mm, vertical = 8.0 mm, lateral = 0.5 mm (shell); anterior = 10.6 mm, vertical = 7.0 mm, lateral = 1.2 mm (core); anterior = 8.6 mm, vertical = 3.5 mm, lateral = 6 mm (ventrolateral striatum). The cannulas directed at the shell were angled 21° from the mid-sagittal plane and those directed at the core 18° from this plane to avoid the ventricular system. The injection volume which was 0.2 μl per side unless otherwise indicated, was delivered over a 20-s period, and the needle was left in situ for an additional 20-s period after completion of the injection. Damage to the target site was minimized by implanting the tips of the guide cannulas 1.2 mm (core), 1.6 mm (ventrolateral striatum) or 2.0 mm (shell) above the desired injection site. Wire stylets were placed in the guide cannulas to prevent occlusion. After surgery, the animals were maintained under anaesthesia by continuous infusion of ketamine (10 mg/h i.v.). Lignocaine HCl (2%) gel was applied to all incisions and the rectal temperature was maintained at 37°C with a thermostatically controlled heating pad. Monitored concentrations of expired O_2 and CO_2 during experiment were 19–21% and 2.0–2.5%, respectively. All experiments were performed according to institutional and national guidelines for animal experimentation.

2.2. Drugs

The animals ($n = 6$–8 per experiment) received bilateral injections of the full dopamine D_1 receptor agonist SKF 82958 (1 or 5 μg; (±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diol hydrobromide; Research Biochemicals International), the dopamine D_2 receptor agonist quinpirole (10 μg; Research Biochemicals International), the putative dopamine D_3 receptor agonist 7-OH-DPAT (5 or 10 μg; (±)-7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin hydrobromide; Research Biochemicals International) or their combination (cocktail). In a separate set of experiments another putative dopamine D_3 receptor agonist PD 128,907 (10 μg; *S*(+)-(4*aR*,10*bR*)-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-[1]-benzopyrano[4,3-*b*]-1,4-oxazin-9-ol hydrochloride; Research Biochemicals International) was tested. Control animals received the solvent of the drugs. In additional sets of experiments the dopamine D_1 receptor antagonist SCH 23390 (500 ng; *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride; Research Biochemicals International) and/or the dopamine D_2 receptor antagonist domperidone (50 or 100 ng; Research Biochemicals International) were given into the shell 10 min prior to the agonist injection. Injection of the dopamine $D_{2/3}$ receptor antagonist *l*-sulpiride (25 ng; Ravizza) with or without SCH 23390 (500 ng) was given 30 min prior to the cocktail. Domperidone and *l*-sulpiride were dissolved in a few drops of diluted acetic acid and then diluted with saline (0.9% w/v NaCl solution) for injection. *l*-Sulpiride was dissolved in 0.5 μl since our previous study has shown that *l*-sulpiride remains ineffective when dissolved in 0.2 μl (Cools et al., 1995). All remaining drugs were dissolved in saline immediately before use. Doses and time schedule were based on previously published studies (Koshikawa et al., 1990a; Prinssen et al., 1992; Cools et al., 1995). Animals were used only once.

2.3. 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 μm) 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.

2.4. Data analysis

All values are expressed as means ± S.E.M. and analysed 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 $P < 0.05$.

3. Results

3.1. Effects of combined injection of SKF 82958 and 7-OH-DPAT into the core and the shell of the nucleus accumbens

Fig. 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 region were discarded in the analysis ($n = 97$). The overall effects of saline, SKF 82958, 7-OH-DPAT and their combinations are shown in Fig. 2. The cocktail of SKF 82958 (5 μg) and 7-OH-DPAT (10 μg) was ineffective, when given into the core (Fig. 2): the

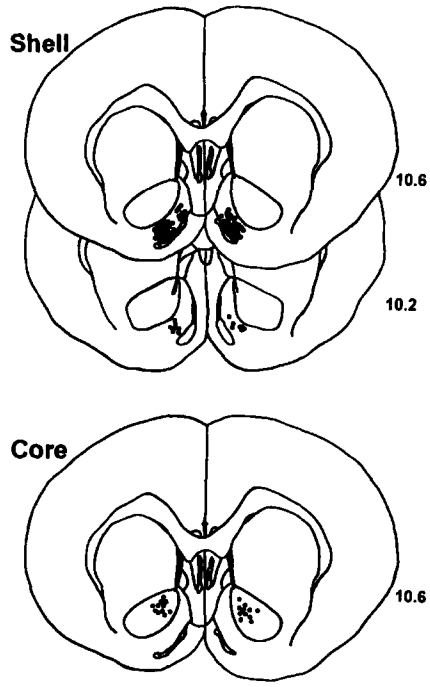

Fig. 1. All injection sites found in the shell (upper part) and the core (lower part) of the nucleus accumbens. Planes are modified to a series of 2 or 3 sections for each brain area from the atlas of Paxinos and Watson (1986); approximate coordinates indicated are in mm anterior to the interaural line.

number of jaw movements in the treated rats ($n = 7$) did not significantly differ from that in solvent treated animals ($n = 6$) [$F(1,11) = 0.94$, $P = 0.34$]. In contrast, this cocktail was highly effective when injected into the shell ($n = 8$), whereas combinations of smaller doses (1 μg SKF 82958 + 10 μg 7-OH-DPAT, $n = 6$ and 5 μg SKF 82958 + 5 μg 7-OH-DPAT, $n = 6$) did not result in any increase in jaw movements. When given alone into the shell, SKF 82958 (5 μg) and 7-OH-DPAT (10 μg) remained also ineffective: the number of jaw movements in the SKF 82958-treated ($n = 6$) and the 7-OH-DPAT-treated

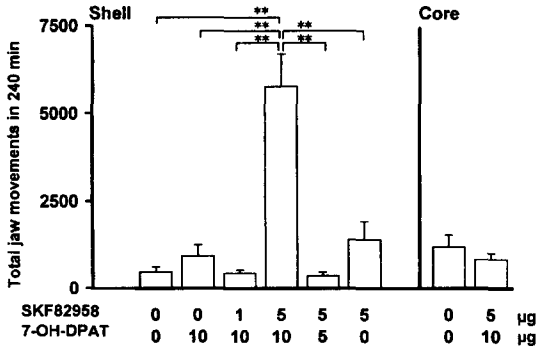

Fig. 2. Effects of injections of saline, SKF 82958, 7-OH-DPAT and the mixture of SKF 82958 and 7-OH-DPAT into the shell (left) and the core (right) of the nucleus accumbens (0.2 μl/side) on production of jaw movements. The data are expressed as the mean number of total jaw movements occurring in a 240-min observation period after injection ($n = 6$–8). Vertical bars indicate S.E.M. ** $P < 0.01$ (Newman-Keuls test).

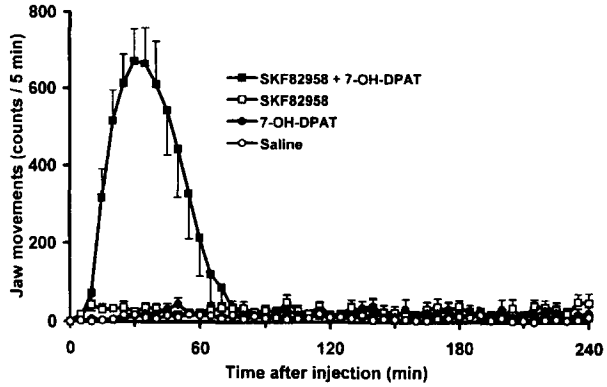

Fig. 3. The time-dependent effects of injections of saline (○), 5 μg SKF 82958 (□), 10 μg 7-OH-DPAT (●) and the mixture of SKF 82958 and 7-OH-DPAT (■) into the shell of the nucleus accumbens on production of jaw movements. The data are expressed as the mean number of jaw movements occurring in 5-min observation periods ($n = 6$–8). Vertical bars indicate S.E.M.

rats ($n = 6$), respectively, did not significantly differ from that found in solvent-treated rats ($n = 7$). Comparing the effects of the effective cocktail with the effects of each single drug resulted in a highly significant difference [overall group effect $F(5,33) = 17.67$, $P < 0.001$; Newman-Keuls $P < 0.01$ vs. SKF 82958, 7-OH-DPAT and solvent, respectively] (Fig. 2). The time-dependent effects of the effective cocktail of SKF 82958 and 7-OH-DPAT are shown in Fig. 3. This figure clearly illustrates that the effect started nearly immediately after the injection, reached its peak around 30 min and vanished about 70 min after injection.

3.2. Effects of SCH 23390 and/or domperidone on jaw movements elicited by administration of the cocktail of SKF 82958 and 7-OH-DPAT into the shell

Injections of SCH 23390 (500 ng, $n = 6$) into the shell significantly, but not completely, attenuated the shell effects of the cocktail of SKF 82958 and 7-OH-DPAT ($F(1,12) = 7.84$, $P < 0.05$; Fig. 4). Injections of domperidone (50 or 100 ng, $n = 6$, respectively) into the shell also significantly attenuated the shell effects of cocktail of SKF 82958 and 7-OH-DPAT (vs. 50 ng, $F(1,12) = 4.37$, $P = 0.058$; vs. 100 ng, $F(1,12) = 8.05$, $P < 0.02$; Fig. 4); again, the effects were only partially antagonized. In contrast, injection of a mixture of these dopamine D_1 and D_2 receptor antagonists ($n = 6$) completely abolished the shell effects of SKF 82958 (5 μg) and 7-OH-DPAT (10 μg) cocktail ($F(1,12) = 19.40$, $P < 0.001$; Fig. 4).

3.3. Effects of SCH 23390 and/or l-sulpiride on jaw movements elicited by administration of the cocktail of SKF 82958 and 7-OH-DPAT into the shell

Injections of the dopamine $D_{2/3}$ receptor antagonist *l*-sulpiride (25 ng, $n = 6$) into the shell tended to attenuate

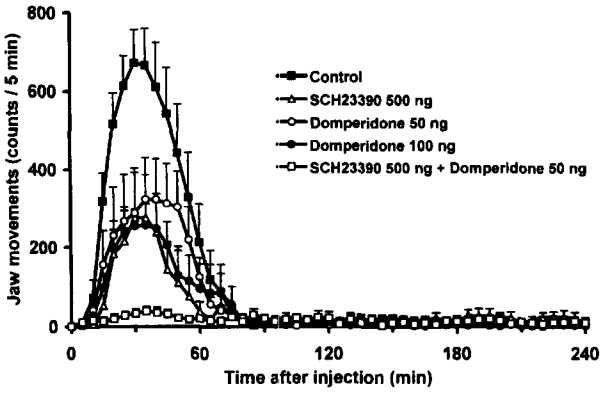

Fig. 4. Effects of SCH 23390 (500 ng, △), domperidone (50 ng, ○ or 100 ng, ●) or the mixture (□) of SCH 23390 (500 ng) and domperidone (50 ng) given 10 min prior to the mixture of SKF 82958 (5 μg) and 7-OH-DPAT (10 μg): all injections were made into the shell of the nucleus accumbens (0.2 μl/side). The data are expressed as the mean number of jaw movements occurring in 5-min observation periods (n = 6–8). Vertical bars indicate S.E.M. Control (■) = effects of the mixture of SKF 82958 and 7-OH-DPAT.

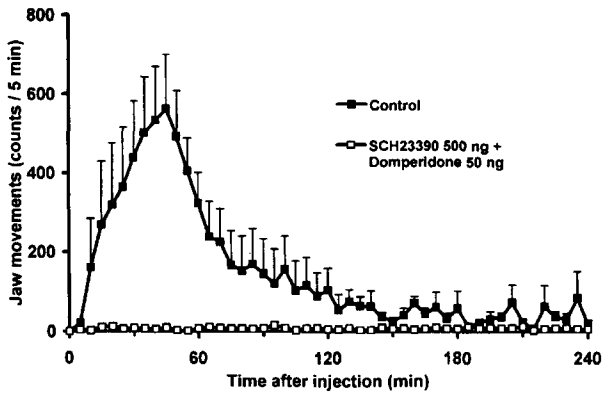

Fig. 6. Effects of the mixture (□) of SCH 23390 (500 ng) and domperidone (50 ng) given 10 min prior to the mixture of SKF 82958 (5 μg) and quinpirole (10 μg): all injections were made into the shell of the nucleus accumbens (0.2 μl). The data are expressed as the mean number of jaw movements occurring in 5-min observation periods (n = 7). Vertical bars indicate S.E.M. Control (■) = effects of the mixture of SKF 82958 and quinpirole (n = 6).

the shell effects of the cocktail of SKF 82958 and 7-OH-DPAT ($F(1,12) = 3.25$, $P = 0.09$; Fig. 5). However, when this dose of *l*-sulpiride was combined with 500 ng SCH 23390 (n = 7), the shell effects of SKF 82958 (5 μg) and 7-OH-DPAT (10 μg) were completely suppressed ($F(1,12) = 23.19$, $P < 0.001$; Fig. 5).

3.4. Effects of combined injection of SCH 23390 and domperidone on jaw movements elicited by administration of the cocktail of SKF 82958 and quinpirole into the shell

The mixture of SCH 23390 (500 ng) and domperidone (50 ng, n = 7) which completely inhibited the shell effects of the cocktail of SKF 82958 and 7-OH-DPAT (see section 3.2.), also produced a complete disappearance of the jaw movements induced by the cocktail of SKF 82958 (5 μg) and quinpirole (10 μg, n = 6; Fig. 6) ($F(1,576) = 122.27$, $P < 0.001$, two-way ANOVA).

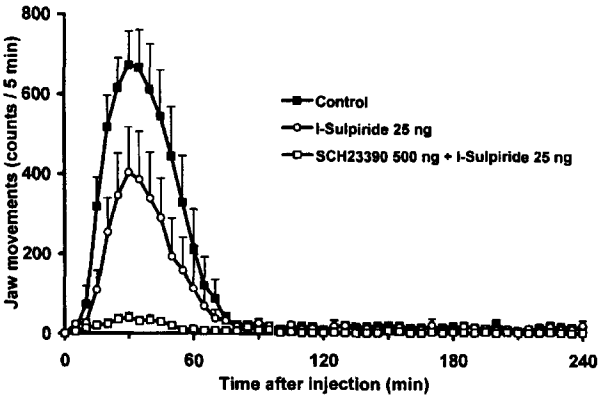

Fig. 5. Effects of *l*-sulpiride (25 ng/0.5 μl, ○) or the mixture (□) of SCH 23390 (500 ng) and *l*-sulpiride (25 ng) given 30 min prior to the mixture of SKF 82958 (5 μg) and 7-OH-DPAT (10 μg): all injections were made into the shell of the nucleus accumbens. The data are expressed as the mean number of jaw movements occurring in 5-min observation periods (n = 6–8). Vertical bars indicate S.E.M. Control (■) = effects of the mixture of SKF 82958 (5 μg) and 7-OH-DPAT (10 μg).

3.5. Effects of administration of the cocktail of SKF 82958 and 7-OH-DPAT into the ventrolateral striatum

Injection of SKF 82958 (5 μg) and 7-OH-DPAT (10 μg) into the ventrolateral striatum (n = 6) produced a highly significant induction of jaw movements ($F(1,576) = 79.35$, $P < 0.001$, two-way ANOVA). The time-dependent effects of the combined injection of SKF 82958 and 7-OH-DPAT into the ventrolateral striatum are shown in Fig. 7. This figure clearly shows that the effect reached its peak around 60–90 min and subsided about 120 min after injection.

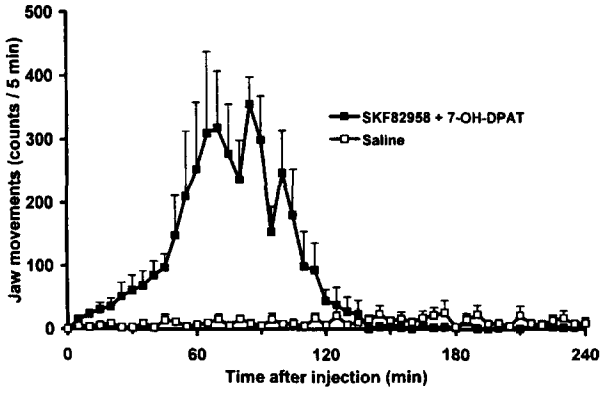

Fig. 7. Effects of injections of saline (□, n = 7) and the mixture (■, n = 6) of SKF 82958 (5 μg) and 7-OH-DPAT (10 μg) into the ventrolateral striatum (0.2 μl). The data are expressed as the mean number of jaw movements occurring in 5-min observation periods. Vertical bars indicate S.E.M.

3.6. Effects of administration of the cocktail of SKF 82958 and PD 128,907 into the shell

Injection of SKF 82958 (5 μg) and the putative dopamine D_3 receptor agonist PD 128,907 (10 μg) into the shell of the nucleus accumbens remained ineffective. The total number of jaw movements elicited during a 240-min observation period being 394.5 ± 113.8 (mean ± S.E.M., $n = 6$); [$F(1,11) = 0.15$, $P = 0.70$] compared to 470.9 ± 151.5 ($n = 7$) after saline.

4. Discussion

The present study and the one reported in the accompanying paper (Koshikawa et al., 1996b) intended to provide evidence that dopamine D_3 receptors play a role in mediating functions of mesolimbic structures as has been suggested before (Diaz et al., 1995; Sokoloff et al., 1990). For that purpose, we chose two behaviours, namely jaw movements (present study) and turning behaviour (accompanying paper: Koshikawa et al., 1996b). These behaviours are known to be dopamine-dependent and specific for the shell region of the nucleus accumbens (Cools et al., 1995; Koshikawa et al., 1996a), namely a mesolimbic structure known to contain a high amount of dopamine D_3 receptors in comparison with the adjacent core region of the same nucleus (Diaz et al., 1995). Furthermore, we chose 7-OH-DPAT as putative dopamine D_3 receptor agonist as tool to manipulate the dopamine receptors in the ventrolateral striatum and core, respectively, shell of the nucleus accumbens. On the basis of the present data we reach the conclusion that the putative dopamine D_3 receptor agonist 7-OH-DPAT is rather a dopamine D_2 receptor agonist than a dopamine D_3 receptor agonist, at least in our in vivo studies. Furthermore, we reach the conclusion that the dopamine D_3 receptor is certainly not involved in the limbic functions under study. Below, the data giving rise to these conclusions are discussed as follows: first, arguments for involvement of dopamine D_3 receptors are given and, next, arguments against the involvement of these receptors are given.

4.1. Arguments for involvement of the dopamine D_3 receptor

First, exchanging the dopamine $D_{2/3}$ receptor agonist quinpirole with the putative dopamine D_3 receptor agonist 7-OH-DPAT in the cocktail with the dopamine D_1 receptor agonist SKF 82958 produced a full blown display of jaw movements when a particular dose combination was administered into the shell; administration of this dose-combination into the core produced nearly no jaw movements. Since the shell has a very high density of dopamine D_3 receptors in comparison with the core (Sokoloff et al., 1990), these data appear to underline the putative involvement of dopamine D_3 receptors. However, the earlier reported finding that only the shell, but not the core, of the nucleus accumbens is involved in the display of jaw movements (Cools et al., 1995) weakens the strength of this argument. Still, comparison of the effects of the two cocktails with 7-OH-DPAT and quinpirole, respectively, shows that the peak of the time-response curve of the cocktail of SKF 82958 and 7-OH-DPAT is higher and more skewed than that of the time-response curve of the cocktail of SKF 82958 and quinpirole (cf. Figs. 1 and 6). Moreover, the data forming the former curve show far less variance than those forming the latter curve. Both sets of data may be taken as evidence in favour of the hypothesis that the selectivity of 7-0H-DPAT at the dopamine D_3 receptor is greater than that of quinpirole at this receptor, a finding which would fit in with earlier reported data (Daly and Waddington, 1993; Damsma et al., 1993; Kreiss et al., 1995; Rivet et al., 1994; Sokoloff et al., 1990; Starr and Starr, 1995; Svensson et al., 1994; Waters et al., 1993). However, differences in physicochemical properties of 7-OH-DPAT and quinpirole may also have contributed to the differences noted.

Second, both the dopamine D_1 receptor antagonist SCH 23390 and the dopamine D_2 receptor antagonist domperidone could only partially inhibit the effects of the cocktail of SKF 82958 and 7-OH-DPAT. Since the doses used were found to antagonize completely the dopamine-dependent and shell-specific turning behaviour (Cools et al., 1995; Koshikawa et al., 1996a), these data open the possibility that the jaw movements which were still present following the inhibition of dopamine D_1 or D_2 receptors, might have been due to the involvement dopamine D_3 receptors. Given the lack of available dopamine D_3 receptor antagonists, however, a direct proof for this hypothesis could not be provided.

4.2. Arguments against the involvement of dopamine D_3 receptors

First, the drug *l*-sulpiride which has a high affinity not only at the dopamine D_2 receptor, but also at the dopamine D_3 receptor (Sokoloff et al., 1990) did not completely antagonize the effects of the cocktail of SKF 82958 and 7-OH-DPAT despite the fact that the dose used is sufficient for inhibiting completely the dopamine-dependent and shell-specific turning behaviour (Koshikawa et al., 1996a). However, combining it with the dopamine D_1 receptor antagonist SCH 23390 was sufficient to antagonize completely all effects elicited by the cocktail.

Second, the combined administration of the dopamine D_1 receptor antagonist SCH 23390 and the dopamine D_2 receptor antagonist domperidone completely antagonized the effects of the cocktail of SKF 82958 and 7-OH-DPAT as well as those of the cocktail of SKF 82958 and quinpirole, excluding thereby the possible involvement of dopamine D_3 receptors in the cocktail effects.

Third, administration of the cocktail of either SKF 82958 and 7-OH-DPAT or SKF 82958 and quinpirole was highly effective in eliciting jaw movements when applied into the ventrolateral striatum, namely a region which contains nearly no dopamine D_3 receptors (Sokoloff et al., 1990).

Fourth, the cocktail of SKF 82958 and another putative dopamine D_3 receptor agonist PD 128,907, which was reported to be far more selective for dopamine D_3 receptors than 7-OH-DPAT (Akunne et al., 1994; Sautel et al., 1995), had no effect at all, when administered into the shell in a dose (10 μg) that 7-OH-DPAT was found highly effective.

All these data together give rise to two conclusions. First, the contribution of 7-OH-DPAT in the cocktail of SKF 82958 and 7-OH-DPAT to the display of jaw movements is solely due to its ability to activate dopamine D_2 receptors. This conclusion fits in with earlier reports showing that this drug behaves similarly to dopamine D_2 receptor agonists (Caine et al., 1995; Freedman et al., 1994; Gonzalez and Sibley, 1995; Large and Stubbs, 1994; Liu et al., 1994; Starr and Starr, 1995). The present study, however, shows that this holds also true for intracerebrally administered 7-OH-DPAT. Second, the dopamine-dependent jaw movements which can be elicited from the shell of the nucleus accumbens or the ventrolateral striatum, are just mediated by dopamine D_1 and D_2 receptors.

In summary, mesolimbic dopamine D_3 receptors play no role in the dopamine-dependent and shell-specific jaw movements. The present study implies that a thorough reevaluation of the dopamine D_3 receptor specificity of 7-OH-DPAT is required.

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