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Intra-accumbens infusion of D_3 receptor agonists reduces spontaneous and dopamine-induced locomotion

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Abstract

The present study investigated whether PD 128907 and 7-OH-DPAT, described as preferential dopamine (DA) D_3 receptor agonists, produce hypolocomotion by acting at postsynaptic dopaminergic receptors within the nucleus accumbens. Bilateral infusion of PD 128907 (1.5 and 3 μg/0.5 μl) induced a dose-dependent hypolocomotion, whereas its enantiomer, PD 128908, was inactive. Local infusion of 7-OH-DPAT and the preferential DA autoreceptor agonist, B-HT 920, at the same dose range also decreased spontaneous locomotion. In addition, both drugs induced yawning with B-HT 920 producing the greatest effect. In the second experiment, the ability of these agonists to reduce the locomotor activity induced by intra-accumbens injection of DA (10 μg/0.5 μl) was studied. Pretreatment with either PD 128907 or 7-OH-DPAT (3 μg) reduced DA-induced hyperactivity. Local infusion of B-HT 920 (3 μg) failed to antagonise the locomotor effects of DA. Altogether these findings suggest that PD 128907 and 7-OH-DPAT induce hypolocomotion by acting in part at postsynaptic DA receptors. The possible role of D_2 and/or D_3 receptors in the mediation of these effects is discussed.

Keywords: Dopamine receptors; Hypolocomotion; Nucleus accumbens; Rat

1. Introduction

Among the newly discovered dopamine (DA) receptors, the D_3 receptor subtype has been of particular interest, in part because of its distinctive pattern of localisation in the brain. The D_3 receptor is predominantly expressed in limbic regions of the brain (e.g., nucleus accumbens, island of Calleja, and hippocampus) known to be associated with cognitive and emotional functions [9,21,23]. This neuroanatomical distribution together with its high affinity for antipsychotic drugs suggests that D_3 receptors may be important targets for the development of therapeutic treatments for schizophrenia and drug abuse [32,36,37]. In the past few years, several existing compounds were reported to display a greater selectivity for D_3 receptors as compared to D_2 receptors. For instance, both PD 128907 and 7-OH-DPAT were characterised as D_3-preferring receptor agonists in both binding and cellular studies, with the former compound being the most selective [5,23,33,35]. However, given systematically, both PD 128907 and 7-OH-DPAT were found to produce behavioural changes (e.g., hypolocomotion and yawning) comparable to those observed with other DA receptor agonists such as apomorphine. Interestingly, unlike apomorphine or other related agonists, the sedative effects induced by systemic administration of 7-OH-DPAT have been reported to occur independently from changes of DA release or synthesis [40]. Similarly, PD 128907 was also found to inhibit amphetamine-induced hyperactivity at doses that had no effect on the increase of extracellular DA levels induced by amphetamine in the ventral striatum [10]. On the basis of these observations, it has been suggested that the hypolocomotion induced by the PD 128907 and 7-OH-DPAT may be mediated via D_3 postsynaptic DA receptors [10,40]. In line with these findings, subsequent studies have shown that PD 128907 could also reduce stereotypies induced by direct DA receptor agonist, apomorphine, and the NMDA receptor antagonist, MK 801. Furthermore, the effects of PD 128907 on MK 801-induced stereotypies

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could be prevented by coadministration of the selective D_3 antagonist, NGB 2900 [43].

Although there is strong evidence that PD 128907 and 7-OH-DPAT suppress locomotion by acting at postsynaptic DA receptors, there have been few attempts to explore the neuroanatomical substrate underlying such action. To address this issue we studied the effects of direct infusion of PD 128907 and 7-OH-DPAT to the nucleus accumbens, a brain structure where D_3 receptors are highly expressed [39]. For comparison we also studied the DA D_2 agonist, B-HT 920 [35]. This compound has been described as a preferential presynaptic DA autoreceptor agonist on the basis of behavioural and neurochemical studies in intact animals [6,28,34]. For instance, B-HT 920 produces hypolocomotion over a wide dose range. Furthermore, unlike direct dopaminergic agonists (e.g., apomorphine and lisuride), high doses of B-HT 920 fail to increase locomotion, suggesting that this compound has only weak agonistic actions at postsynaptic D_2 receptors [3,28]. This apparent selectivity for presynaptic autoreceptors has been related to the difference of receptor reserves in dopaminergic neurones and their target neurones [14,25].

The first part of the study was designed to characterise the behavioural effects induced by local infusion of PD 128907, 7-OH-DPAT, and B-HT 920 into the nucleus accumbens. To further investigate the possibility that a population of postsynaptic DA receptors might mediate suppression of locomotion, we have studied the ability of these agonists to reduce the hyperactivity induced by bilateral intra-accumbens infusions of DA. Since the hyperlocomotor activity induced by DA is mediated by postsynaptic DA receptors, suppression of DA's effect would suggest that D_3-preferring agonists act via postsynaptic mechanisms to reduce locomotion.

2. Methods

2.1. Subjects

Male Sprague–Dawley rats (Zivic-Miller) weighing 280–300 g at the time of surgery were housed two per cage with free access to food and water. They were kept on a standard day–night cycle (6 a.m.–6 p.m.) under temperature-controlled conditions. Animals were treated in strict accordance with guidelines set forth in the PHS manual (Guide for the Care and Use of Laboratory Animals).

2.2. Surgery and infusion procedure

The surgery was performed under xylazine (0.07 mg/kg) and ketamine (25.5 mg/kg) anaesthesia. The rats were implanted with a double-guide cannula (22 gauge, Plastics One) positioned 3 mm above the nucleus accumbens at the coordinates: AP: +1.7 mm from the bregma, L ±1.5 mm from midline, DV −4.5 mm from the skull surface with the incisor bar set at −3.2 mm, according to the atlas of Paxinos and Watson [30]. The cannulae were fixed to the skull with dental cement and stainless-steel screws. A double dummy cannula was inserted into the guide cannula to prevent occlusion and a dust cap was used to secure the unit to the double-guide cannula. One week after implantation, a double internal cannula (28 gauge) was inserted through the double-guide cannula to 3 mm beyond its tip and a volume of 0.5 μl was infused at a rate of 0.16 μl/min with Hamilton syringe mounted on a microdrive pump. Following the injection, the needle remained in place for another 2 min to allow the drug to diffuse away from the injection site. Each subject was used only once.

2.3. Drugs

DA was dissolved in 0.9% saline solution containing ascorbic acid (0.1 mg/ml) as antioxidant. PD 128907 [*S*-(+)-*trans*-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano[4,3-*b*]-1,4-oxazin-9-ol]; PD 128908 [*R*(−)-(4*aS*,10*bs*)-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano-[4,3-*b*]-1,4-oxazin-9-ol]; 7-OH-DPAT [(+)-2-dipropylamino-7-hydroxy-1,2,3,4-tetrahydronaphthalene]; and B-HT 920 [5,6,7,8-tetrahydro-6-(2-propenyl)-4*H*-thiazolo[4,5-*d*]azepin-2-amine] were dissolved in 0.9% saline at pH 6.5–7. All drugs were obtained from RBI, Natick, MA.

2.4. Behavioural testing

Locomotor activity was recorded using automated activity cages (40 × 40 × 30 cm) in a room that was masked with white noise and maintained at 25°C. Each cage was fitted with 12 infrared beams (4 front to back and 8 side to side) located 5 cm off the cage floor (San Diego Instruments). Noncumulative records of photocell beam interruptions, for individual rats, were taken every 5 min in each experimental session. The incidence of yawning was scored continuously during the first 30-min period of the test in blocks of 5-min intervals for each animal. Yawning was defined as vertical opening of the mouth for a prolonged period of time (i.e., >1 s).

2.4.1. Experiment I: novelty-induced locomotor exploration

Groups of animals received bilateral injections of PD 128907 (1.5 and 3 μg/0.5 μl, $n=7$ and 8, respectively), 7-OH-DPAT (1.5 and 3 μg/0.5 μl, $n=7$ and 8, respectively), or B-HT 920 (1.5 and 3 μg/0.5 μl, $n=7$ per dose) into the nucleus accumbens. Another group received a bilateral injection of PD 128908 (3 μg/0.5 μl, $n=7$), the inactive enantiomer of PD 128907. Corresponding control groups for each treatment received a bilateral infusion of physiological saline solution (NaCl 0.9%, $n=7$) into the nucleus accumbens. Immediately after administration of the drugs, spontaneous locomotor activity was monitored for 60 min. The animals were tested in groups of 3–4 and they were

continuously observed for the first 30-min period of the test by a person blind to drug treatments.

2.4.2. Experiment II: DA-induced locomotor hyperactivity

First group of animals received bilateral intra-accumbens injections of DA (10 μg/0.5 μl) immediately after microinjection of physiological saline ($n=7$) or PD 128907 (3 μg/0.5 μl, $n=8$). A second group of animals received a bilateral infusion of DA immediately after infusion of physiological saline ($n=7$) or 7-OH-DPAT (3 μg/0.5 μl, $n=8$). The third group of animals received a bilateral infusion of DA immediately after infusion of physiological saline ($n=8$) or B-HT 920 (3 μg/0.5 μl, $n=8$). A control group received an injection of physiological saline alone into the nucleus accumbens ($n=6$). The animals were first familiarised with the experimental cages during a 2-h habituation period. After habituation, the compounds were administered to the animals and their locomotor activity was immediately recorded for 60 min. Direct observation of the behaviour of the animals was performed during the first 30-min period of the test by a person blind to drug treatments.

2.5. Histology

After completion of behavioural testing, animals were killed by decapitation and their brains rapidly removed and frozen by slow immersion into isopentane cooled by dry ice. Brain sections were cut at a thickness of 20 μm with a Harris cryostat (-17°C) and stained with Cresyl violet to check the accuracy of the injection sites.

2.6. Statistical analysis

Behavioural data for each agonist in the dose response experiments were analysed by a one-way ANOVA with dose of the agonists as the between subject factor. The effects of the agonists on locomotion were analysed over the whole 60-min period. The effects of the agonists on the incidence of yawning were analysed over the first 30-min period of the test. The effects of the agonists on DA-induced locomotion were analysed by a one-way ANOVA over the whole 60-min period with pretreatment as the between subject factor. Post hoc comparisons were carried out using Fisher PLSD test. The significance level was taken to be $P<.05$.

3. Results

3.1. Histological verification

Fig. 1 illustrates the area of placements accepted as falling within the nucleus accumbens. The histological analysis showed that most of the injection sites for the subjects of Experiments I and II fell within the nucleus accumbens in the anterior planes +1.7 to +1 mm from the bregma according to the atlas of Paxinos and Watson [30]. Eight subjects in which the injection sites were located outside of the nucleus accumbens were discarded from statistical analysis.

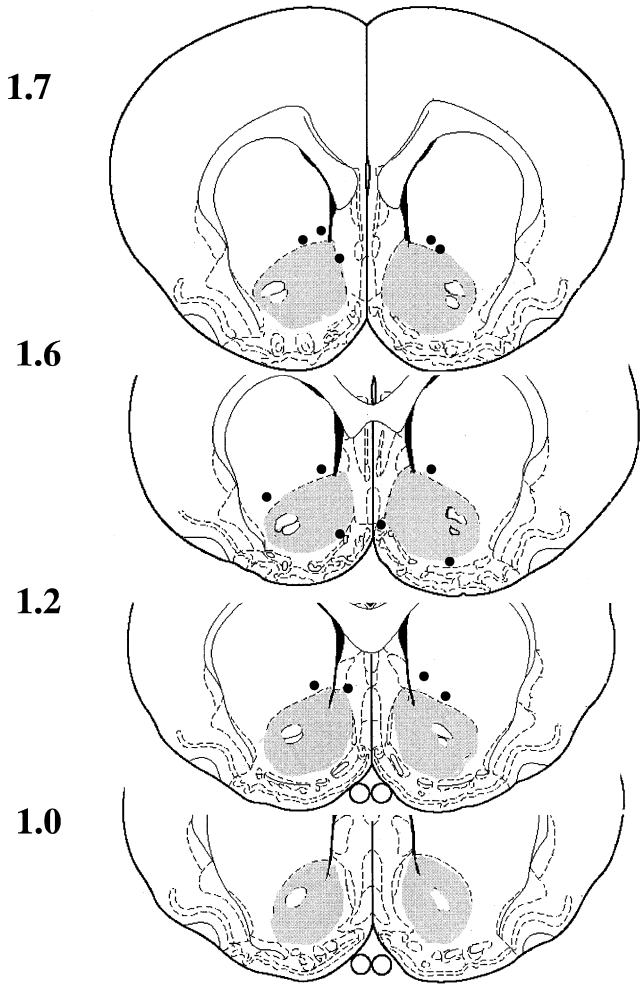

Fig. 1. Diagrammatic representation of coronal sections (1.7 to 1.0 mm anterior to bregma) through the rat brain showing the area of placements accepted as falling within the nucleus accumbens (shaded). Placements falling outside the target area are shown by filled circles marking the tip of the injection needle (data from these rats were excluded from analysis).

3.2. Effects of D_3-preferring agonists on novelty-induced locomotor exploration

As shown in Fig. 2, the spontaneous locomotor activity of the animals treated with intra-accumbens injection of saline solution was initially high and then rapidly decreased as the animals habituated to their environment. Bilateral intra-accumbens infusion of PD 128907 (1.5 and 3 μg/0.5 μl) significantly decreased spontaneous locomotor activity [$F(2,20)=6.04$, $P<.01$; Fig. 2]. As compared to the control group, only the effect of the highest dose was statistically significant ($P<.05$ Fisher PLSD, graph insert). To test for the potential nonspecific effect of local injec-

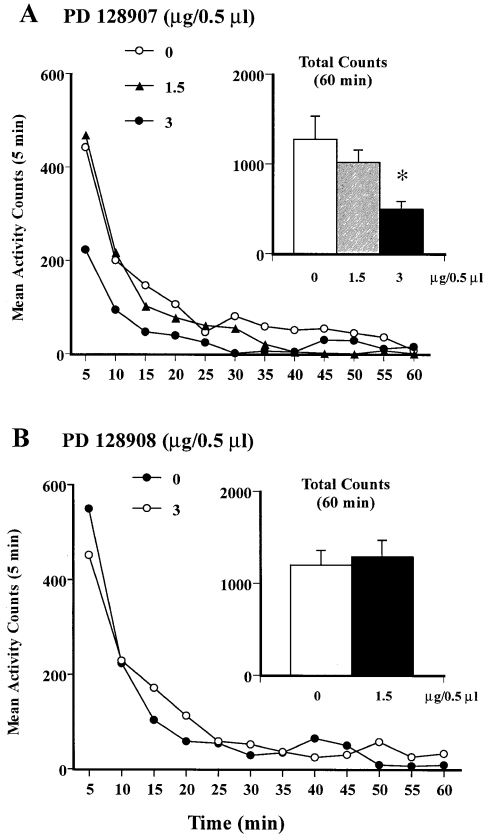

Fig. 2. Effect of intra-accumbens infusion of PD 128907 (A) and its enantiomer PD 128908 (B) on spontaneous locomotion. (A) The animals received a bilateral infusion of PD 128907 (1.5 and 3 μg/0.5 μl, $n=6$ and 8, respectively) or physiological saline solution (NaCl 0.9%, $n=7$) into the nucleus accumbens. (B) The animals received a bilateral injection of PD 128908 (3 μg/0.5 μl, $n=7$) or saline solution ($n=7$) into the nucleus accumbens. Ordinates give the mean number of photocell counts in 5-min periods. Inserts: Mean locomotor activity counts (±S.E.M.) during the entire 60-min period. * Significantly different from controls, $P<.05$, Fisher PLSD after significant ANOVA.

tions on spontaneous locomotor activity, PD 128908, the less active enantiomer of PD 128907, was tested. As shown in Fig. 2B, bilateral intra-accumbens infusion of PD 128908 at the high dose (3 μg/0.5 μl) failed to reduce spontaneous locomotion.

Bilateral injection of 7-OH-DPAT (1.5 and 3 μg/0.5 μl) into the nucleus accumbens produced significant reduction of spontaneous locomotion [$F(2,19)=4.23$, $P<.05$; Fig. 3A]. Post hoc comparison indicates that only the effect of the highest dose (3 μg) differed significantly from saline treatment during the overall 60-min test period ($P<.05$, Fisher PLSD test, graph insert). As shown in Fig. 3B, intra-accumbens infusion of B-HT 920 (1.5 and 3 μg/0.5 μl) also markedly reduced spontaneous locomotor activity [$F(2,19)=3.57$, $P<05$]. Post hoc comparisons indicate that only the animals treated with the highest dose had significantly a lower spontaneous locomotor activity than control animals ($P<.05$, Fisher PLSD test, graph insert).

3.3. Direct observation of rat's behaviour

Fig. 4, illustrates the effects of local infusion of PD 128907, 7-OH-DPAT, and B-HT 920 on the incidence of yawning during the first 30-min period following the injection. PD 128907 induced occasional yawning at both doses tested (1.5 and 3 μg/0.5 μl). However, these effects were not statistically significant at any of the doses tested [$F(2,20)=1.99$, $P>.05$; Fig. 4A]. Animals treated with intra-accumbens infusion of PD 128908 (3 μg) did not show yawning (data not shown). Direct injection of 7-OH-DPAT (1.5 and 3 μg) into the nucleus accumbens markedly increased yawning. One-way ANOVA indicated a significant main effect [$F(2,19)=7.7$, $P<.01$; Fig. 4B], and post hoc comparisons showed that both doses of 7-OH-DPAT significantly increased yawning as compared to the control group ($P<.05$, Fisher PLSD test). Intra-accumbens infusion of B-HT 920 at the same dose range

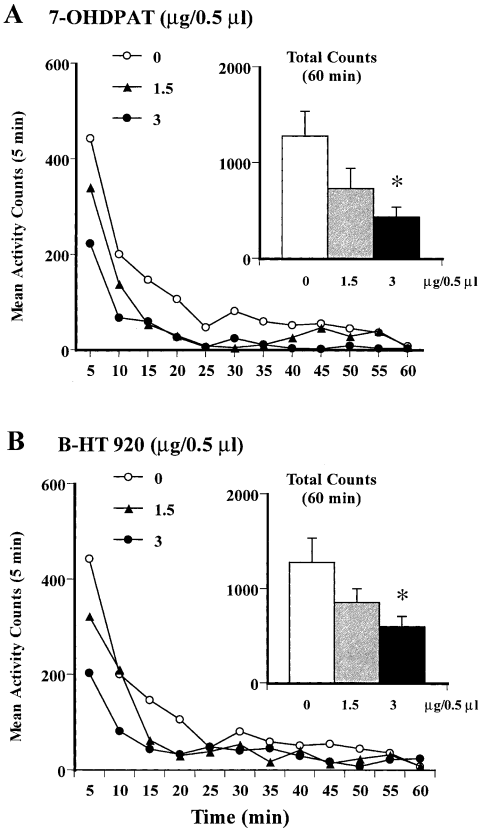

Fig. 3. Effect of intra-accumbens infusion of 7-OH-DPAT (A) and B-HT 920 (B) on spontaneous locomotion. (A) The animals received 7-OH-DPAT (1.5 and 3 μg/0.5 μl, $n=6$ and 7, respectively) or a physiological saline solution (NaCl 0.9%, $n=7$) bilaterally in the nucleus accumbens. (B) The animals received B-HT 920 (1.5 and 3 μg/0.5 μl, $n=6$ and 7, respectively) or a physiological saline solution (NaCl 0.9%, $n=7$) bilaterally in the nucleus accumbens. Ordinates give the mean number of photocell counts in 5-min periods. Inserts refer to mean total locomotor activity counts (±S.E.M.) during the entire 60-min test. * Significantly different from controls, $P<.05$, Fisher PLSD after significant ANOVA.

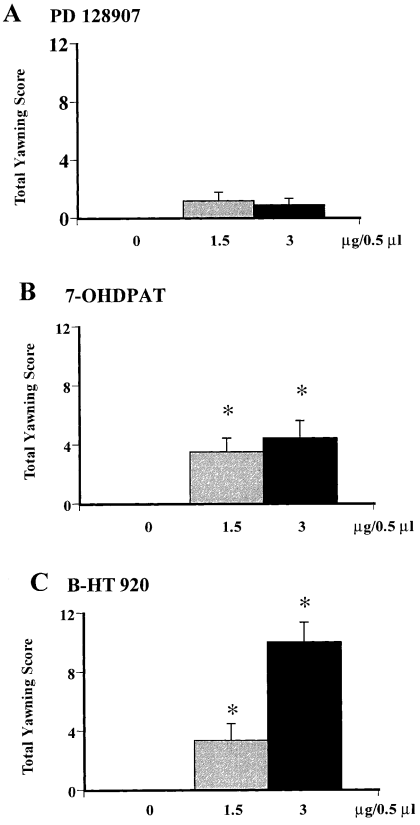

Fig. 4. Effect of intra-accumbens infusion of PD 128907 (A), 7-OH-DPAT (B) and B-HT 920 (C) on yawning. (A) The animals received PD 128907 (1.5 and 3 μg/0.5 μl, $n=6$ and 8, respectively) or a physiological saline solution (NaCl 0.9%, $n=7$) bilaterally in the nucleus accumbens. (B) The animals received 7-OH-DPAT (1.5 and 3 μg/0.5 μl, $n=6$ and 7, respectively) or a physiological saline solution (NaCl 0.9%, $n=7$) bilaterally in the nucleus accumbens. (C) The animals received B-HT 920 (1.5 and 3 μg/0.5 μl, $n=6$ and 7, respectively) or a physiological saline solution (NaCl 0.9%, $n=7$) bilaterally in the nucleus accumbens. Ordinates refer to mean total yawning scores (±S.E.M.) during the first 30 min of the test. *Significantly different from controls, $P<.05$, Fisher PLSD after significant ANOVA.

into the nucleus accumbens induced a clear dose-dependent increase in yawning. One-way ANOVA revealed a significant main effect [$F(2,19)=27.9$, $P<.001$; Fig. 4C]. Post hoc test indicated that both doses tested significantly increased yawning relative to controls ($P<.05$, Fisher PLSD test).

3.4. Effect of D_3-preferring agonists on DA-induced locomotor hyperactivity

Habituated animals given bilateral combined infusion into the nucleus accumbens of saline, followed by DA (10 μg) solution, showed an increase of locomotor activity in comparison to control animals injected with saline solution alone (Figs. 5 and 6). Observation of the animals after DA infusion showed that the raised activity was due to increased ambulation associated with intensive sniffing and occasional rearing. In keeping with this trend, overall ANOVA revealed a significant main effect [$F(2,20)=6.25$, $P<.05$] and post hoc test indicated that animals injected with DA had significantly higher level of locomotor activity as compared to control animals injected with saline (Fig. 5A). Prior administration of PD 128907 (3 μg) significantly reduced the locomotor hyperactivity induced by DA ($P<.05$, Fisher PLSD test). Similar suppression of DA-induced locomotor activity was seen after pretreatment with 7-OH-DPAT (3 μg) (Fig. 5B). Overall ANOVA revealed a significant main effect [$F(2, 19)=12.41$, $P<.001$], and post hoc test indicated that animals injected with 7-OH-DPAT had significantly lower levels of locomotor activity as compared to the one treated with DA alone (Fig. 5B, $P<.05$ Fisher PLSD test). Prior administration of B-HT 920 at the same dose

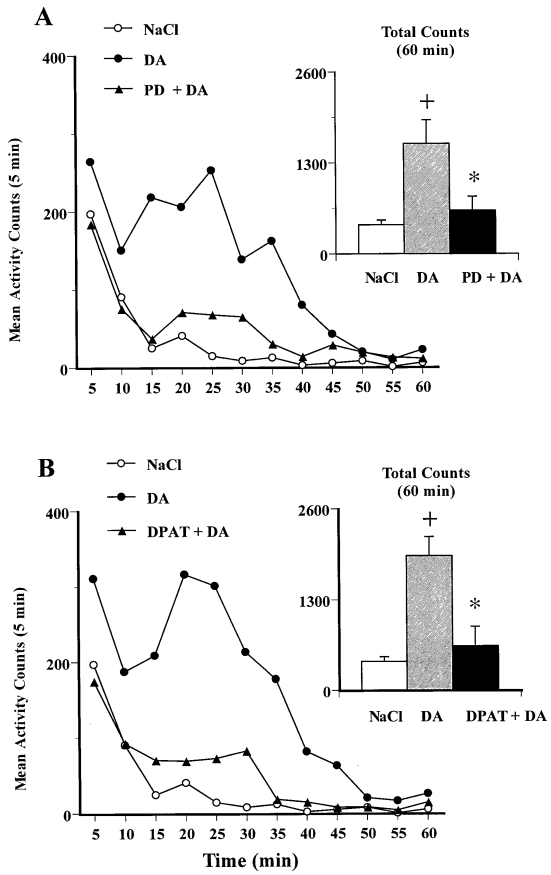

Fig. 5. Effect of intra-accumbens infusion of PD 128907 (A) and 7-OH-DPAT (B) on the locomotor hyperactivity induced by DA. (A) The animals received an intra-accumbens injection of PD 128907 (3 μg/0.5 μl, $n=7$) followed immediately by an injection of DA (10 μg/0.5 μl). (B) The animals received an intra-accumbens injection of 7-OH-DPAT (3 μg/0.5 μl, $n=7$) followed immediately by an injection of DA (10 μg/0.5 μl). Control groups for DA received intra-accumbens injection of saline solution followed immediately by an injection of DA ($n=8$). Another control group ($n=6$) received bilateral injection of saline solution only into the nucleus accumbens. Ordinates give the mean number of photocell counts in 5-min periods. Inserts refer to mean total locomotor activity counts (±S.E.M.) during the entire 60-min test. +Significantly different from saline-treated group. *Significantly different from DA-treated group, $P<.05$, Fisher PLSD after significant ANOVA.

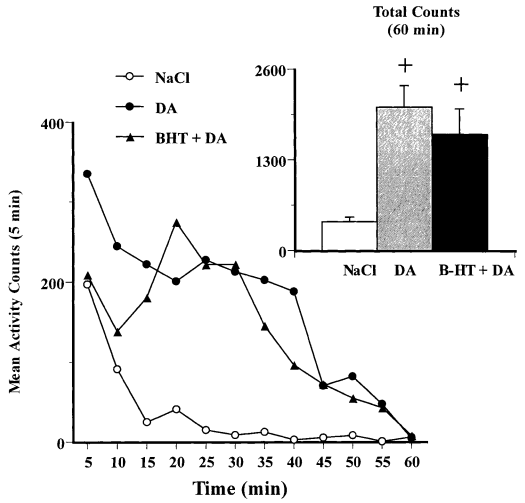

Fig. 6. Effect of intra-accumbens infusion of B-HT 920 on the locomotor hyperactivity induced by DA. The animals received an intra-accumbens injection of B-HT 920 (3 μg/0.5 μl, $n=6$) followed immediately by an injection of DA (10 μg/0.5 μl). A control group for DA received intra-accumbens injection of saline solution followed immediately by an injection of DA ($n=8$). Another control group ($n=6$) received bilateral injection of saline solution only into the nucleus accumbens. Ordinates give the mean number of photocell counts in 5-min periods. Insert refers to mean total locomotor activity counts (±S.E.M.) during the entire 60-min test. $^{+}$Significantly different from saline-treated group, $P<.05$, Fisher PLSD after significant ANOVA.

failed to reduce significantly the locomotor hyperactivity induced by DA (Fig. 6).

4. Discussion

The present behavioural study shows that all three DA agonists, PD 128907, 7-OH-DPAT, and B-HT 920, injected into the nucleus accumbens reduced spontaneous locomotor activity. However, only 7-OH-DPAT and B-HT 920 increased the incidence of yawning. More importantly, these three agonists differentially suppressed the locomotor hyperactivity induced by local infusion of DA into the nucleus accumbens. Whilst both PD 128907 and 7-OH-DPAT markedly reduced DA-induced locomotor hyperactivity, local infusions of the putative DA autoreceptor agonist, B-HT 920, failed to antagonise the effects of DA. These findings indicate that the nucleus accumbens is one of the brain areas mediating yawning and hypolocomotion induced by systemic administration of low doses of dopaminergic agonists. Furthermore, they suggest that a population of postsynaptic DA receptors within the nucleus accumbens may be involved in mediating the locomotor suppression induced by PD 128907 and 7-OH-DPAT.

Previous studies have shown that low doses of 7-OH-DPAT injected into the nucleus accumbens reduced the locomotor activity of rats tested in a novel environment [13,19]. Our study confirms these previous findings and further demonstrates that local injection of the D_3-preferring agonist, PD 128907, as well as the preferential DA autoreceptor agonist, B-HT 920, can also induce hypolocomotion. Interestingly, we have found that the three agonists tested showed a marked difference in their ability to induce yawning. Indeed, a clear increase in the incidence of yawning was seen after both injections of 7-OH-DPAT and B-HT 920, with the latter inducing the greatest effect. However, microinjection of PD 128907 at the same dose range failed to elicit yawning (but the possibility exists that a higher dose might be effective). Thus, in the yawning test 7-OH-DPAT displayed a behavioural profile more comparable to B-HT 920 than PD 128907. It is unlikely that this behavioural difference between PD 128907 and 7-OH-DPAT reflects differences in bioavailability or intrinsic activity. In fact, at the higher dose tested (3 μg) both drugs induced a comparable reduction of locomotor activity (during the first 30 min, PD 128907 reduced locomotor activity by 39% and 7-OH-DPAT by 42% relative to the control). The present finding would suggest that the differential effects of PD 128907 and 7-OH-DPAT on yawning might be due to an action on distinct receptors. Several binding and functional in vitro studies have shown that 7-OH-DPAT has a modest selectivity for D_3 versus D_2 receptors than PD 128907 [12,35]. It is therefore possible that populations of presynaptic D_2 receptors may mediate yawning induced by 7-OH-DPAT. In support of this suggestion the autoreceptor agonist B-HT 920, which had the greatest effect on yawning, was shown to display a higher functional selectivity toward D_2 receptor subtypes [35]. However, we cannot exclude the possibility that 7-OH-DPAT and B-HT 920 may induce yawning by acting on other receptors. For instance, B-HT 920 has also high affinity for α_1-adrenergic receptors, which have been implicated in the modulation of yawning [18].

To further investigate the possibility that a population of postsynaptic DA receptors may mediate suppression of locomotion, we have studied the ability of PD 128907, 7-OH-DPAT, and B-HT 920 to reduce the hyperactivity induced by bilateral intra-accumbens infusion of DA. In line with previous studies, local infusion of DA into the nucleus accumbens caused a marked locomotor hyperactivity in rats [2,29,31,41]. Interestingly, both PD 128907 and 7-OH-DPAT markedly reduced the locomotor activity induced by intra-accumbens injection of DA; in contrast, the putative DA autoreceptor agonist, B-HT 920, was ineffective. The lack of effect of B-HT 920 suggests that (1) the suppression of DA-induced locomotor hyperactivity is not due to a potential nonspecific effect resulting from combined injection of the drugs and (2) that the D_3-preferring agonists reduce DA effects by acting at postsynaptic rather than presynaptic receptors. Consistent with this finding, systemic administration of PD 128907 was found to inhibit amphetamine-induced locomotor hyperactivity in rats at doses that had no effect on the increase of extracellular DA level induced by amphetamine in the ventral striatum [10]. More

importantly, PD 128907 also reduced the hyperactivity induced by the D_1 receptor agonist SKF 81297, as well as stereotypies induced by the direct DA receptor agonist apomorphine, and the NMDA receptor antagonist MK 801 [27,43]. Furthermore, the effects of PD 128907 on MK 801-induced stereotypies could be prevented by coadministration of selective D_3 antagonist NGB 2900 [43]. Given that PD 128907 possesses a higher affinity for the D_3 versus D_2 receptors, it is conceivable that its suppressive effect on DA-induced hyperactivity may be due to the stimulation of postsynaptic D_3 receptors. In line with this suggestion, It was recently shown that the ontogeny of motor inhibition induced by low doses of PD 128907 coincides in time with the developmental expression of DA D_3 receptors within the nucleus accumbens [15,36]. Further evidence for the role of D_3 receptors in the inhibition of locomotor activity is also provided by several studies using more selective strategies such as the antisense knockdown approach. Indeed, intracerebroventricular infusion of an antisense oligodeoxynucleotide directed against D_3 receptor subtypes mRNA reduced D_3 receptor density in the nucleus accumbens and increased spontaneous locomotor activity in rat [11,45]. Furthermore, D_3 antisense oligodeoxynucleotide treatment increased locomotor activity induced by apomorphine in DA-depleted rats [26], thus revealing the role of postsynaptic D_3 receptors in the inhibition of locomotor activity. It is important to stress that these effects were observed with different sequences of D_3 antisense oligodeoxynucleotides, thus confirming the selectivity of the antisense knockout approach. Moreover, we found that the D_2 antisense oligodeoxynucleotide induces an opposing effect on locomotion [45]. Altogether, these findings strongly suggest that D_2 and D_3 receptors regulate the expression of locomotor behaviour in rat in an opposing direction.

It should be noted, however, that recent data from gene knockout studies have questioned the role of D_3 receptors in the suppression of locomotion. While some authors have reported increased locomotion in mice lacking the D_3 receptor gene [1,44], others failed to observe these effects [8]. Furthermore, only mice lacking D_2, but not D_3 receptor genes were found to be nonresponsive to the hypolocomotion induced by 7-OH-DPAT and PD 128907, thus suggesting that these compounds may be rather acting on D_2 receptors to inhibit locomotion [7]. As recently demonstrated by Levin [22], D_3 receptors are differentially expressed in certain brain regions in mice and rats, and it is thus possible that they may also differentially regulate certain behaviours in these two species. It should be also stressed that there are clear phenotypic differences between D_3 mutant mice generated in different laboratories [1,8,38,44], and this seems also the case for D_2 mutant mice [4,7,17]. These discrepancies strongly suggest that the behavioural phenotypes of the mutants are not due only to the loss of the targeted receptors but also to several other factors including developmental adaptations and genetic background [17]. As such, it cannot be excluded that these factors might also influence drug responses [16,24], and thus contribute to the discrepant results obtained in rats and mice.

Finally, it was recently shown that 7-OH-DPAT and PD 128907 act as partial agonist at both D_2 and D_3 receptors [42], but see Ref. [35]. It is therefore possible that these ligands might behave as D_2 receptor agonists or antagonists depending on the level of the dopaminergic tone. For instance, the suppression of spontaneous locomotion may be due to the stimulation of presynaptic $D_{2/3}$ receptors, whereas the blockade of DA-induced locomotor hyperactivity may be related to an antagonism of postsynaptic D_2 receptors. However, this hypothesis seems unlikely, especially for PD 128907. Firstly, in functional assays unlike 7-OH-DPAT, PD 128907 was found to behave as a partial agonist that lacks antagonist properties [42]. Furthermore, pretreatment with the selective D_3 receptor antagonist NGB 2900 was shown to prevent PD 128907-induced blockade of stereotypies evoked by MK 801 [43], which strongly suggests that PD 128907 acts via stimulation of D_3 receptors.

In conclusion, our findings confirm previous studies showing that 7-OH-DPAT and PD 128907 reduce spontaneous and DA agonist-induced locomotion. Furthermore, they suggest that this suppression of locomotion can be mediated via at least two independent mechanisms within the nucleus accumbens: (1) a presynaptic mechanism involving inhibition of DA release, probably via stimulation of D_2 receptor subtypes [20], and (2) a postsynaptic mechanism involving activation of D_2-like receptors. Further studies using intra-accumbens infusions of highly selective D_2 and D_3 receptor ligands are clearly needed to confirm whether the postsynaptic receptors are of D_2 or D_3 subtype.

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