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Ontogeny of the motor inhibitory role of dopamine D_3 receptor subtype in rats

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Abstract

We have examined the motor responses to the dopamine D_3 receptor-preferring agonist, *S*(+)-(4*aR*,10*bR*)-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-1-benzopyranol[4,3-*b*]-1,4-oxazin-9-ol ((+)-PD128,907), by non-habituated male rats during postnatal development. (+)-PD128,907 (0.025 and 0.1 mg/kg) increased motor activity (rearing, motility and locomotion) in 14-day-old rats without inducing oral stereotypies. However, in 21-, 28- and 70-day-old rats, (+)-PD128,907 caused a significant reduction in motor activity. This reduction was most pronounced in 70-day-old rats. In addition, the stimulatory effects of (+)-PD128,907 in 14-day-old rats were fully blocked by the dopamine D_3 receptor antagonist 5,6-dimethoxy-2-(di-*u*-propylamino) indan (U99194A). These results suggest that the motor inhibition mediated by the activation of the dopamine D_3 receptors develops between the second and the third postnatal weeks.

Keywords: Dopamine; Dopamine D_3 receptor; (+)-PD128,907; Motor activity; Postnatal development

1. Introduction

The neurotransmitter, dopamine, plays an important modulatory role on various brain functions such as cognitive, motor, and reward-related behaviours (Le Moal, 1995). Dysfunctions of the mesolimbic and nigrostriatal dopaminergic pathways have been linked to various brain-related pathologies such as schizophrenia. In recent years, attention has focused on the development of these dopaminergic pathways and in particular, on the roles of multiple dopamine receptor subtypes since recent evidence indicates that schizophrenia may have a neurodevelopmental origin (Weinberger, 1995).

Among the newly cloned dopamine D2-like receptors (e.g., D_2, D_3 and D_4 receptor subtypes), the dopamine D_3 receptor subtype has received special attention based on its predominant expression in the ventral part of the striatum, and its high affinity for dopamine and many antipsychotic drugs (see Sokoloff and Schwartz, 1995). This has prompted much research into elucidation of the function of the dopamine D_3 receptors. Several studies have implicated the dopamine D_3 receptor subtype as the receptor that may mediate motor suppression in the adult rat (for a review see Shafer and Levant, 1998). However, little is known about the specific role of the dopamine D_3 receptors in motor activity during postnatal development. A previous study has used the putative dopamine D_3 receptor agonist, 7-OH-DPAT (7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin), to investigate the role of the dopamine D_3 receptors during postnatal development (Frantz et al., 1996). However, the selectivity of this compound for the dopamine D_3 receptor is less than previously thought (Burris et al., 1995). We have, therefore, examined the effects of the preferential dopamine D_3 receptor agonist, (+)-PD128,907 (*S*(+)-(4*aR*,10*bR*)-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-1-benzopyranol[4,3-*b*]-1,4-oxazin-9-ol) (Sautel et al., 1995), on motor activity during postnatal development.

2. Materials and methods

2.1. Subjects

Sprague–Dawley rats were obtained from B&K (Sollentuna, Sweden). Day of birth was considered as day 0.

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The animals were separated from their dams at 25 days of age. They were kept in a temperature and humidity controlled room under a regular light/dark cycle (lights on at 0600 h and off at 1800 h) with free access to food pellets and tap water. Eight rats aged 14, 21, 28 or 70 days were randomly assigned to each dose group. Testing took place between 0900 and 1500 h. All animals were allowed to rest for at least 4 days after arrival until used in the experiment. All the experiments were carried out with non-habituated animals that were used only once to avoid carry-over effects.

2.2. Drug procedure

(+)-PD128, 907 hydrochloride (Research Biochemical and International, Natrick, MA, USA) was dissolved in saline (0.9% NaCl), which was also used as the vehicle. Subcutaneous (s.c.) injections were administered into the neck in doses of 0.025 and 0.1 mg/kg in a volume of 1 ml/kg.

2.3. Behavioural procedure

Motor activity was measured simultaneously in eight animals by means of a multicage detection system (Ögren et al., 1979). Briefly, this uses beams of red and infrared light in combination with vertical and horizontal (4 cm distance between cells) photocell arrays to detect movements of animals. Rearing was measured by counting the number of times an animal stood on its hindlegs and interfered with any of the six invisible infrared beams passing horizontally through the cages. The height of these photocells was adapted to the size of the animal. Motility was measured by counting all movements over a distance of 4 cm as detected by vertical photocells, and represents a measurement of general activity. Locomotion was measured by counting the number of times an animal moved from one side of the box to another (a distance of at least 32 cm). Immediately after the injection, each animal was placed in the center of an activity cage (novel environment). Data were collected for 2-min intervals over a period of 30 min.

2.4. Statistics

Dose-dependent effects of (+)-PD128, 907 on motor activity were analyzed by two-way analysis of variance (ANOVA) for repeated measures for each age group with treatment and time as main factors. If a significant interaction was found, then one-way ANOVA was carried out to

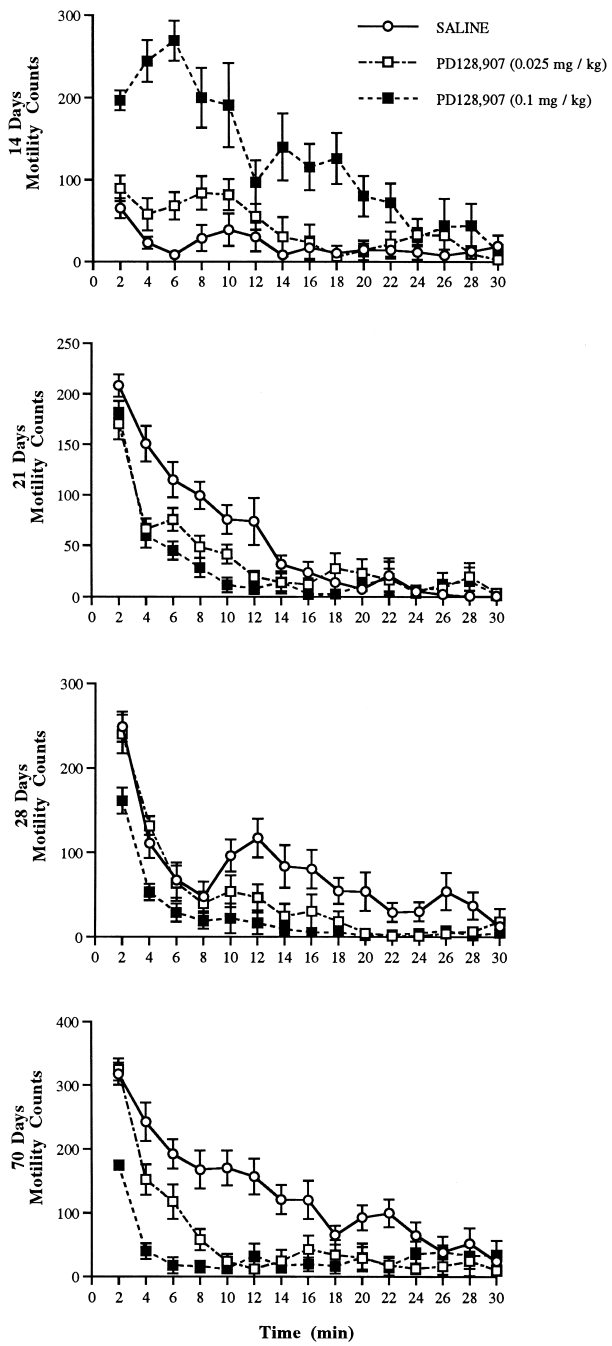

Fig. 1. Time course of the effects of (+)-PD128,907 on motility of non-habituated rats during various postnatal ages (means ± S.E.M.; $n = 8$/group). Significant differences from saline group of the same age group are as follows: In 14-day-old rats the, 0.1 mg/kg dose of (+)-PD128,907 increased activity at the $P < 0.001$ level from 2 to 8 min and 18-min interval and at $P < 0.05$ level during the 10-, 14-, 16-, and 20-min intervals. In 21-day-old rats, the 0.1 mg/kg dose of (+)-PD128,907 decreased activity at the $P < 0.001$ level from 4 to 10 min and at the $P < 0.05$ level at the 12-min interval. The 0.025 mg/kg dose decreased activity at the 4- ($P < 0.001$) and from 6- to 12-min intervals ($P < 0.05$). In 28-day-old rats, the 0.1 mg/kg dose decreased activity during the 2, 4, 10 and from the 14- to 28-min intervals at the $P < 0.05$ level and at the $P < 0.001$ level at the 12-min interval. The 0.025 mg/kg decreased activity at the $P < 0.05$ level during the 12- and the 14-min intervals and from 18 to 28 min. In 70-day-old rats, the 0.1 mg/kg dose decreased activity from the 2- to 14-min intervals ($P < 0.001$) and at the 16- and the 12-min intervals ($P < 0.05$). The 0.25 mg/kg dose decreased activity from 8- to 14-min intervals ($P < 0.001$) and at the 4-, 6-, 16-, and 22-min intervals ($P < 0.05$).

test the effect of drug dose at each interval. The post-hoc Fisher's least significant difference (LSD) test was used for subsequent analysis of significance at the $P < 0.05$ or $P < 0.001$ level.

3. Results

Since the overall time-curve pattern for the rearing, motility and locomotion parameters was similar for the various ages studied, only the time-curve for motility is presented (Fig. 1). The data accumulated over the 30-min recording period for the various ages and parameters studied are summarized in Fig. 2.

3.1. 14-day-old rats

In 14-day-old rats, two-way ANOVA for repeated measures revealed a significant effect of (+)-PD128, 907 on rearing [$F(2,21) = 6.714$, $P = 0.0056$], motility [$F(2,21) = 15.303$, $P < 0.0001$] and locomotion [$F(2,21) = 10.737$, $P = 0.0006$]; significant time-effect for rearing [$F(14,294) = 3.536$, $P < 0.0001$], motility [$F(14,294) = 18.007$, $P < 0.0001$] and locomotion [$F(14,294) = 8.051$, $P < 0.001$]; and significant dose × time interaction for rearing [$F(28,294) = 2.074$, $P = 0.0016$], motility [$F(28,294) = 7.086$, $P < 0.0001$] and locomotion [$F(28,294) = 4.011$, $P < 0.001$]. The post-hoc Fisher's LSD test, made for each time-point separately, revealed that the higher dose (0.1 mg/kg) of (+)-PD128,907 tested produced significant increases (with respect to vehicle-injected control group) in rearing, motility and locomotion (see Figs. 1 and 2). The lower dose (0.025 mg/kg) induced only a short stimulation that did not reach significance. The hypermotility observed after (+)-PD128.907 (0.1 mg/kg) was characterized by exploration of the activity box without signs of oral stereotypies (e.g., chewing, biting, licking).

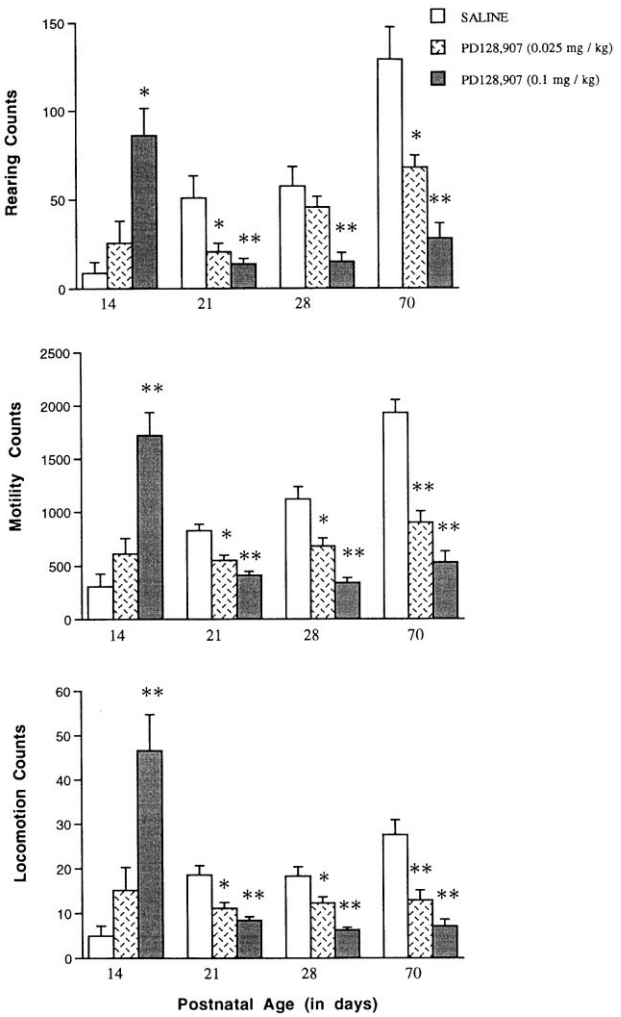

Fig. 2. The effects of (+)-PD128,907 on motor activity of 14-, 21-, 28- and 70-day-old rats. The accumulated counts (30-min testing period) for rearing, motility and locomotion of non-habituated rats for various postnatal ages (means ± S.E.M.; $n = 8$/group). Significant differences from saline group of the same age group are shown: $^{*}P < 0.05$, $^{**}P < 0.001$.

To establish whether the behavioural activation induced by (+)-PD128,907 in 14-day-old rats was mediated by the dopamine D_3 receptor, a new set of animals was injected with the dopamine D_3 receptor agonist, 5,6-dimethoxy-2-(di-*u*-propylamino) indan (U99194A), (Pharmacia-Upjohn, Kalamazoo, MI, USA), or saline in their home cages. Fifteen minutes later, they were injected with (+)-PD128,907 (0.1 mg/kg) or saline and placed in the activity box as described above. In agreement with the first experiment, (+)-PD128,907 (0.1 mg/kg) significantly increased motor activity (rearing, motility and locomotion; see Fig. 3). Pretreatment with the U99194A compound (3 mg/kg, s.c.) completely antagonized the motor activation induced by (+)-PD128,907 (0.1 mg/kg) in 14-day-old rats (Fig. 3).

3.2. 21-day-old rats

In contrast to the 14-day-old rats, both the high (0.1 mg/kg) and the low (0.025 mg/kg) doses of (+)-PD128,907 decreased rearing, motility and locomotor activity in this age group (see Figs. 1 and 2). Behavioural suppression was characterized by a frozen-like position. A significant effect of dose × time interaction for rearing [$F(28,294) = 3.246$, $P < 0.0001$], motility [$F(28,294) = 3.579$, $P < 0.0001$] and locomotion [$F(28,294) = 1.743$, $P = 0.0133$] as well as significant dose and time effects on rearing [$F(2,21) = 7.716$, $P = 0.0031$] and [$F(14,294) = 12.550$, $P < 0.0001$], respectively, motility [$F(2,21) = 15.431$, $P < 0.0001$] and [$F(14,294) = 66.512$, $P < 0.0001$], respectively, and locomotion [$F(2,21) = 10.330$, $P = 0.0008$] and [$F(14,294) = 54.620$, $P < 0.0001$], respectively, were found in this age group.

3.3. 28-day-old rats

Similarly to the effect in the 21-day-old rats, both the high and low dose of (+)-PD128, 907 decreased rearing motility and locomotion of this age group (see Figs. 1 and

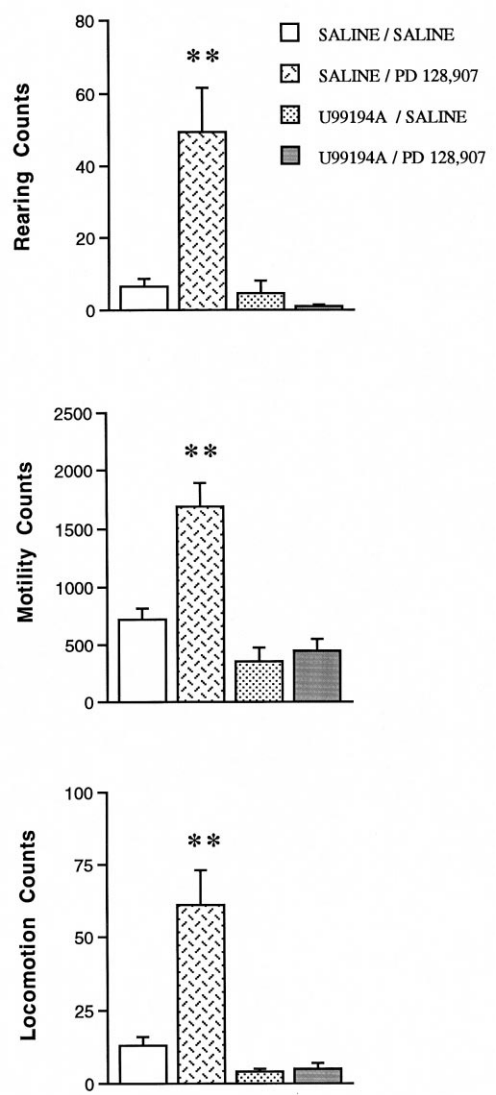

Fig. 3. The effects of U99194A compound on motor stimulation produced by (+)-PD128,907 in 14-day-old rats. Animals received a single s.c. injection of U99194A compound (3 mg/kg) or saline (1 ml/kg); 15 min later, they were injected with (+)-PD128,907 (0.1 mg/kg) or saline and their motor activity was immediately recorded. The accumulated counts (30-min testing period) for rearing, motility and locomotion are presented (means ± S.E.M.; $n = 6–8$/group). Significant differences from saline group of the same age group are shown: $^{**}P < 0.001$.

2). In 28-day-old rats there was a significant effect of (+)-PD128,907 on rearing, motility and locomotion [$F(2,21) = 7.956$, $P = 0.0027$], [$F(2,21) = 21.103$, $P < 0.0001$] and [$F(2,21) = 16.236$, $P < 0.0001$], respectively, and a significant time effect [$F(14,294) = 29.894$, $P < 0.0001$], [$F(14,294) = 44.485$, $P < 0.0001$] and [$F(14,294) = 76.527$, $P < 0.0001$, respectively, as well as a significant ($P < 0.001$) dose × drug interaction for rearing and motility.

3.4. 70-day-old rats

In 70-day-old rats, both doses of (+)-PD128,907 decreased rearing, motility and locomotion (see Figs. 1 and 2). A significant effect of dose × time interaction for rearing [$F(28,294) = 4.037$, $P < 0.0001$], motility [$F(28,294) = 4.717$, $P < 0.0001$] and locomotion [$F(28,294) = 2.790$, $P < 0.0001$] as well as significant dose and time effects on rearing [$F(2,21) = 17.279$, $P < 0.0001$] and [$F(14,294) = 17.569$, $P < 0.0001$], respectively, motility [$F(2,21) = 42.306$, $P < 0.0001$] and [$F(14,294) = 35.984$, $P < 0.0001$], respectively, and locomotion [$F(2,21) = 17.460$, $P < 0.0001$] and [$F(14,294) = 29.137$, $P < 0.0001$], respectively, were found for this age group.

4. Discussion

The main finding of this study was that (+)-PD128,907, a preferential dopamine D_3 receptor agonist, exerts differential motor effects during ontogeny. In 14-day-old rats, (+)-PD128,907 produces motor stimulation, while in 21-, 28- and 70-day-old rats it induces hypomotility. These results suggest that the locomotor inhibition mediated by the activation of the dopamine D_3 receptors develops between the second and the third postnatal week (see below).

The mesolimbic dopaminergic pathway is known to participate in the control of spontaneous activity and dopamine-mediated motor behaviours (see Le Moal, 1995). In particular, descending effects from the nucleus accumbens via the ventral pallidum to mesencephalic locomotor region provide a link between limbic and motor regions (Mogenson, 1991). The dopamine D_3 receptors are highly expressed in the limbic regions (mainly islands of Calleja and nucleus accumbens) of the adult rat (Sokoloff and Schwartz, 1995). Several pharmacological and molecular studies have implicated the dopamine D_3 receptor subtype as the receptor that may mediate motor suppression in the rat (for a review see Shafer and Levant, 1998).

In view of the above, the lack of motor inhibition after (+)-PD128,907 in the 14-day-old rats could be explained by the fact that functional inhibitory dopamine D_3 receptors in the nucleus accumbens may not be present at this age. This possibility is supported by results of two recent studies that have examined the ontogeny of the dopamine D_3 receptors in the rat forebrain (Stanwood et al., 1997; Gurevich et al., 1999). Thus, the dopamine D_3 receptors are slightly expressed in the nucleus accumbens during the first 2 weeks after birth, thereafter increasing their expression with age. In contrast, dopamine D_3 receptor expression in the islands of Calleja reach near adult levels by the second postnatal week. Importantly, this developmental pattern of dopamine D_3 receptor expression is considerably different from what was seen for the dopamine D_1 and D_2 receptors (Rao et al., 1991).

The motor responses to (+)-PD 128,907 (0.025 and 0.1 mg/kg) obtained in the present study were similar in several aspects to those found even after lower doses of the mixed dopamine D_3/D_2 receptor agonist, 7-OH-DPAT

(0.01 mg/kg), used in a previous study (Frantz et al., 1996). Both drugs induce motor stimulation in animals younger than 3 weeks of age. Moreover, the age at which motor suppression was first observed was similar for both compounds. However, it is clear from the available data that (+)-PD128,907 was more potent to induce hypomotility than 7-OH-DPAT. This is likely to be related to the relative selectivity of these two compounds for the dopamine D_3 vs. the dopamine D_2 receptor subtypes (Sautel et al., 1995).

In contrast, motor suppression induced by a low dose (e.g., 0.1 mg/kg) of the dopamine D_2/D_3 agonist, quinpirole, and the $D_1/D_2/D_3$ agonist, apomorphine (Sautel et al., 1995), was not observed until the fourth week of age (Shalaby and Spear, 1980; Van Hartesveldt et al., 1994). Taken together, these observations suggest multiple mechanisms mediating motor inhibition in the rat. The present results suggest a neuronal substrate involving the dopamine D_3 receptors that is already present at the third postnatal week. The participation of a sub-population of the dopamine D_2 receptors with a later maturation mediating motor inhibition must also be considered.

The high levels of dopamine D_3 receptor mRNA and protein present in the islands of Calleja already at 14 days of age (see above) suggest earlier maturation of these receptors in this area than in the nucleus accumbens. In view of the observation that the dopamine D_3 receptor antagonist, U99194A, was able to fully block the stimulatory effects of (+)-PD128,907 in 14-day-old rats, one may postulate involvement of the dopamine D_3 receptors located in the islands of Calleja in the stimulatory effects of (+)-PD128,907. Further studies are, however, required to explore this possibility.

In conclusion, the ontogeny of motor inhibition induced by low doses of (+)-PD128,907 coincides in time with the developmental expression of the dopamine D_3 receptors within the nucleus accumbens (Stanwood et al., 1997; Gurevich et al., 1999). Therefore, it seems possible that the tonic locomotor inhibition mediated by dopamine D_3 receptor activation is related to the maturation of the dopamine D_3 receptors within the nucleus accumbens in the rat. Thus, there may be a shift (from stimulation to inhibition) in dopamine D_3 receptor function between the second and third postnatal weeks after birth within the ventral striatum.

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