ELSEVIER

Pharmacology, Biochemistry and Behavior 67 (2000) 55–63

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

A9 and A10 dopamine nuclei as a site of action for effects of 8-OH-DPAT on locomotion in the rat

Viveka Hillegaart[a,b,*], Olle Magnusson[b], Sven Ahlenius[a]

[a]*Department of Physiology and Pharmacology, Karolinska Institute, SE-171 77 Stockholm, Sweden*
[b]*Research and Development, AstraZeneca, SE-151 85 Södertälje, Sweden*

Received 21 December 1999; received in revised form 20 March 2000; accepted 27 March 2000

Abstract

The 5-hydroxytryptamine (5-HT) 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) was applied locally (0–5 μg bilaterally) into either the substantia nigra (A9) or the ventral tegmental area (A10) of adult male Wistar rats, and 10 min later spontaneous motor activity was observed in an open field (≈0.5 m^2) for 30 min. The rate of dopamine synthesis was estimated in neostriatal areas, the amygdala, and the prefrontal cortex, by measuring the accumulation of DOPA, following inhibition of cerebral decarboxylase by means of 3-hydroxybenzylhydrazine (NSD-1015). The A10 application of 8-OH-DPAT resulted in an increase in all aspects of spontaneous motor activity in the open field. A9 application of 8-OH-DPAT produced a stereotyped forward locomotion, characterized by a modest decrease in total horizontal activity, almost complete inhibition of rearing activity, and an increase in proportion forward locomotion along the perimeter of the open-field arena. The injection of 8-OH-DPAT into the A9 was accompanied by an increased neostriatal DA rate of synthesis, whereas the A10 injection was followed by a decreased DA rate of synthesis in the amygdala and in the prefrontal cortex. It is concluded that mesencephalic dopaminergic mechanisms are involved in the stereotyped forward locomotion characteristically seen after systemic administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT.

Keywords: Open field; Locomotion; Substantia nigra; Ventral tegmental area; Dopamine; 5-HT_{1A} receptors; Rat

1. Introduction

5-Hydroxytryptamine (5-HT) 5-HT_{1A} receptor agonists, such as 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) [8,17] and flesinoxan [5,11,29], produce a characteristic stereotyped forward locomotion in rats when placed in an open field [2,5,13,15]. This stereotyped locomotion is characterized by slow aimless continuous forward locomotion along the perimeter of the arena. The net effect may be an apparent increase [19,33], or decrease [15,25], in locomotion, depending on how behavioral observations are performed. This pattern of locomotor activity can be pharmacologically distinguished from similar effects produced by, for example, the 5-$HT_{2A/C}$ receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), or the dopamine (DA) $D_{2/3}$ receptor agonist 7-OH-DPAT ([13]; Salmi and Ahlenius, unpublished observations).

It appears that the effects of systemically administered 8-OH-DPAT are due to a complex interaction between autoreceptor-mediated effects in the median and dorsal raphe nuclei [12], as well as postsynaptic 5-HT_{1A} receptors [6]. In addition, both 8-OH-DPAT and flesinoxan also exert effects on brain dopaminergic mechanisms [3,4,31], and these effects are at least partially mediated directly at the DA autoreceptor [7].

The aim of the present study was to investigate to what extent the effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT on spontaneous locomotor activity in the rat are mediated via mesencephalic dopaminergic mechanisms. Thus, in the present study, the effects of 8-OH-DPAT on spontaneous locomotor activity in an open-field arena (≈0.5 m^2) [10] were observed after its local application into the substantia nigra (SN) or into the ventral tegmental area (VTA). In a separate series of experiments, effects of 8-OH-DPAT on forebrain DA synthesis were examined. The

* Corresponding author. Tel.: +46-8-553-254-64; fax: +46-8-553-254-20.
E-mail address: viveka.hillegaart@astrazeneca.com (V. Hillegaart).

0091-3057/00/$ – see front matter
PII: S0091-3057(00)00292-6

rate of DA synthesis was estimated by monitoring the DOPA accumulation following inhibition of cerebral aromatic L-amino acid decarboxylase [9]. The brain areas sampled included neostriatal subdivisions, the amygdala, and the prefrontal cortex.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (280–320 g) were used (B&K Universal AB, Sollentuna, Sweden). The animals arrived in the laboratory at least 10 days before being used in experiments and were housed, five per cage (Makrolon IV), under controlled conditions of temperature (21.0±0.4°C), relative humidity (55–65%), and light–dark cycle (12 L:12 D, lights off 0600 h). Food (R36, Ewos, Södertälje) and tap water were available ad lib in the home cage.

The studies were approved by the Stockholm North Local Ethical Committee on Animal Experiments.

2.2. Drugs

3-Hydroxybenzylhydrazine 2HCl (NSD-1015), mol wt 211.10, (Sigma, St. Louis, MO), and (±)-8-hydroxy-2-(di-*n*-propylamino) tetralin HBr (8-OH-DPAT), mol wt 328.29, (RBI, Natick, MA). Both compounds were dissolved in physiological saline. NSD-1015 was injected intraperitoneally in a volume of 2 ml kg^{-1}. 8-OH-DPAT was administered intracerebrally, in a volume of 0.5 μl, as detailed below.

2.3. Surgery and intracerebral cannulation

The animals were deeply anesthetized with a pentobarbital formulation (Mebumal Vet., Nordvacc, Huddinge, Sweden), 60 mg kg^{-1} IP (1 ml kg^{-1}). The anesthetized rats were mounted in a stereotaxic frame (Stellar, Stoelting, Chicago, IL) and provided with bilateral guide cannula (21 gauge) reaching the dura mater above the intended target. The coordinates for VTA were: −5.3 mm in relation to bregma, ±2.2 mm lateral (the cannulas were tilted 10° in the coronal plane). The corresponding figures for the SN were: −5.3 mm, ±2.4 mm (using a vertical approach). Brain site coordinates were adopted from Paxinos and Watson [27]. The injection needles (31 gauge) reached the respective target 7.8 and 7.6 mm below the brain surface. The animals were allowed 1 week of postoperative recovery before being included in experiments. Immediately after the operation and henceforth, the animals were housed individually in Makrolon III cages. As the animals recovered from surgery, no gross behavioral abnormalities were noted and the animals gained weight rapidly. Thus, the mean weight±SD at the time of surgery was 296±21 g, and the weight at the end of experiments, 3 weeks later, was 334±22 g. All injections were made bilaterally, and the total amount of drug delivered per animal is thus twice the dose given in Figs. 3–5. The rate of injection was 0.33 μl min^{-1}.

Immediately after the final behavioral observation, the animals were injected with 0.5 μl of Evans blue (0.1% in physiological saline). Following the injection, the animals were decapitated, the brain removed, and stored in 40% formalin until sectioned on a microtome. The tissue was inspected under a dissection microscope, and the center of injection site was marked on individual charts prepared from the atlas of Paxinos and Watson [27]. Fifteen out of 15, and 13 of 15 cannulations aimed for the VTA and the SN, respectively, were localized within the borders indicated in Fig. 1. Thus, two animals in the SN cannulation group were excluded in the analysis of results from the open-field experiments.

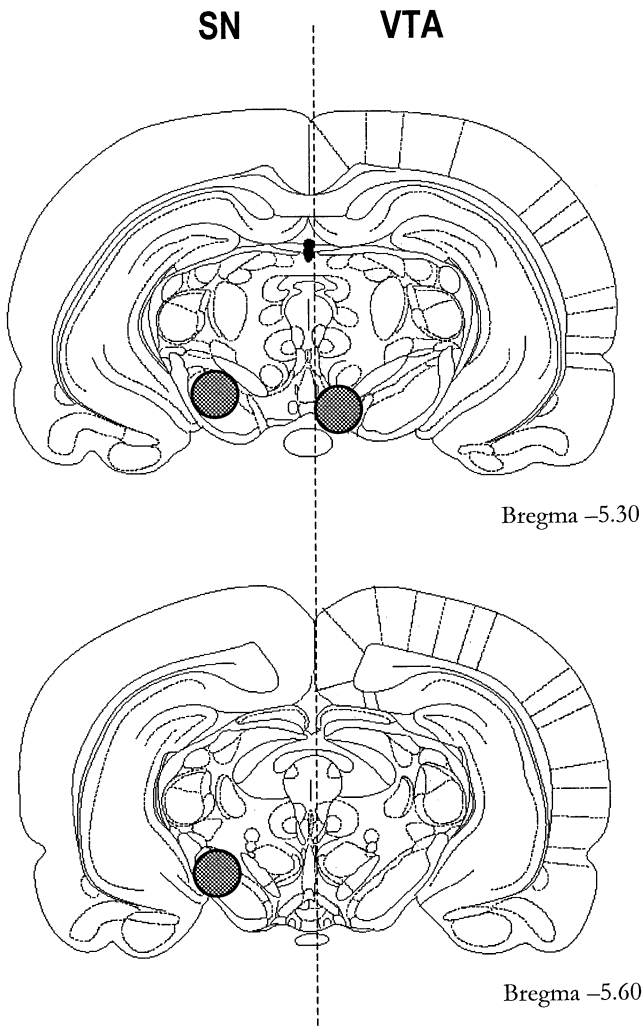

Fig. 1. Schematic presentation of localization of injections into the substantia nigra (SN) (left), and into the ventral tegmental area (VTA) (right), for results presented in Figs. 2–5. Reference brain sections from Paxinos and Watson [27].

2.4. Motor activity observations

The spontaneous motor activity was observed in a square open-field arena (680 × 680 × 450 mm), equipped with two rows of infrared photocells (8×8). Two identical frames of photocells were placed at two levels, 40 and 125 mm above the floor, respectively. The photocells were spaced 90 mm apart, and the last photocell in a row was spaced 25 mm from the wall. The open field was enclosed in a ventilated, sound-attenuating box with a Perspex top. Measurements were made in the dark and performed between 0900 and 1600 h. Each session in the open field lasted for 30 min.

The number of photocell beam interruptions were collected via a PC, and the following variables were calculated: locomotor activity (all interruptions of photobeams at the lower level); peripheral locomotion (interruptions of photobeams provided that the photobeams spaced 25 mm from the wall at the lower level also had been activated); rearing (all interruptions of photobeams at the upper level); forward locomotion (successive interruptions of photocells in the lower rows when the animal was moving in the same direction).

Locomotor activity and rearing data were subjected to a square root transformation. Peripheral locomotion and forward locomotion are expressed as percent of total horizontal activity, and the quotients were based on raw data. For further details on the apparatus and the computer software used, see Ericson et al. [10].

Immediately before being placed in the open field, the animals were scored for the presence of flat body posture.

2.5. Brain dissections and biochemical procedures

Following decapitation, the whole brain (including the olfactory bulb rostrally and the medulla oblongata caudally) was quickly removed and placed in a mold where it could be sliced into 2.5-mm sections with a thin stainless steel wire (=70 μm). The brain was cut at an angle of approximately 7°, such that ventrally the sections extended slightly rostrally, according to the atlas of Paxinos and Watson [27]. The mean weight (mg) ±SD of the ventral neostriatum, the olfactory tubercle, the dorsolateral neostriatum, the amygdala, and the prefrontal neocortex, as dissected here, were 17.1±3.3, 15.4±3.0, 26.4±2.6, 13.1±2.3, and 12.6±2.1, respectively. For further details on the dissection procedures, see Hillegaart et al. [14]. The brain samples were immediately frozen on dry ice and stored at −70°C until processing. DOPA and 5-HTP were determined by means of coupled column liquid chromatography with electrochemical detection. DOPA and 5-HTP levels were quantified with an intra-assay precision of 1–2%. The limit of detection was about 1 pmol sample^{-1} (10–20 pmol g^{-1}). The preparation of the samples and further details are given in Magnusson et al. [24].

2.6. Experimental design and statistics

In the motor activity studies, the animals served as their own controls in a changeover repeated-measurements de-

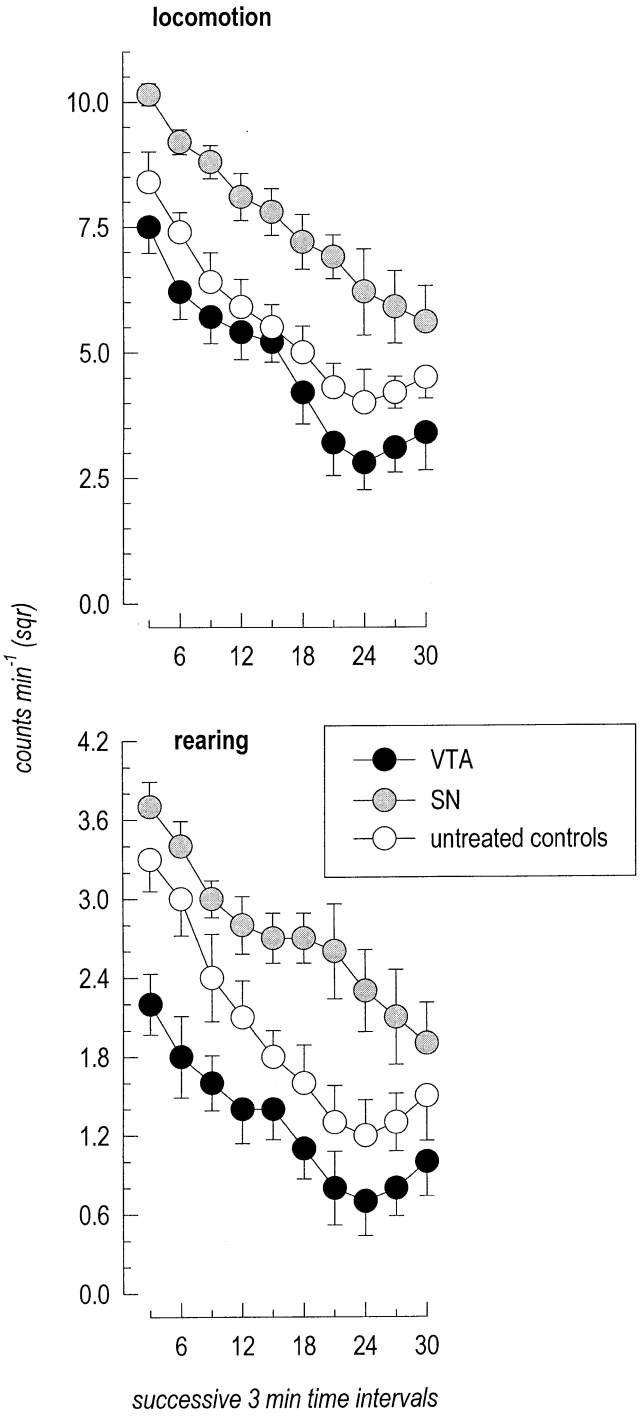

Fig. 2. Effects of saline control injections into the ventral tegmental area (VTA) or the substantia nigra (SN) on locomotion and rearing behaviors. The saline vehicle was injected 10 min before a 30-min session in the open field (cf. Fig. 3A). The figure shows means±SEM based on repeated observations of 15 (VTA), 13 (SN), and 10 untreated rats, respectively.

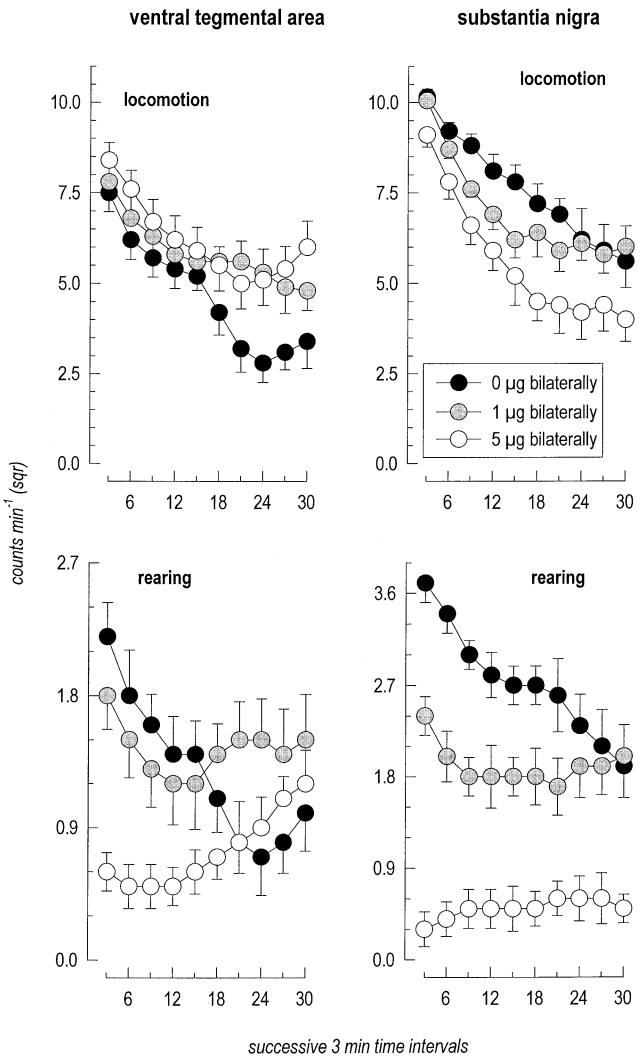

Fig. 3. (A) Effects of bilateral application of the 5-HT_{1A} receptor agonist 8-OH-DPAT into the ventral tegmental area (VTA), or the substantia nigra (SN), on locomotion and rearing in the male rat. 8-OH-DPAT (0–5 µg $side^{-1}$) was administered 10 min before the animals were placed in the open field, and the spontaneous motor activity was monitored for 30 min thereafter. The figure shows means±SEM based on repeated observations of 15 (VTA) and 13 (SN) rats, respectively. Statistical analysis was performed by means of a two-way ANOVA for repeated measurements. Locomotion (VTA), $F(2, 28)=4.93$, $p<0.05$ (dose); $F(9, 126)=31.77$, $p<0.01$ (time); $F(18, 252)=1.63$, $p>0.05$ (dose × time). Rearing (VTA), $F(2, 28)=13.40$, $p<0.01$ (dose); $F(9, 126)=10.89$, $p<0.01$ (time); $F(18, 252)=5.63$, $p<0.01$ (dose × time). Locomotion (SN), $F(2, 24)=7.29$, $p<0.01$ (dose); $F(9, 108)=46.51$, $p<0.01$ (time); $F(18, 216)=1.89$, $p<0.01$ (dose × time). Rearing (SN), $F(2, 24)=91.81$, $p<0.01$ (dose); $F(9, 108)=5.53$, $p<0.01$ (time); $F(18, 216)=3.62$, $p<0.01$ (dose × time).

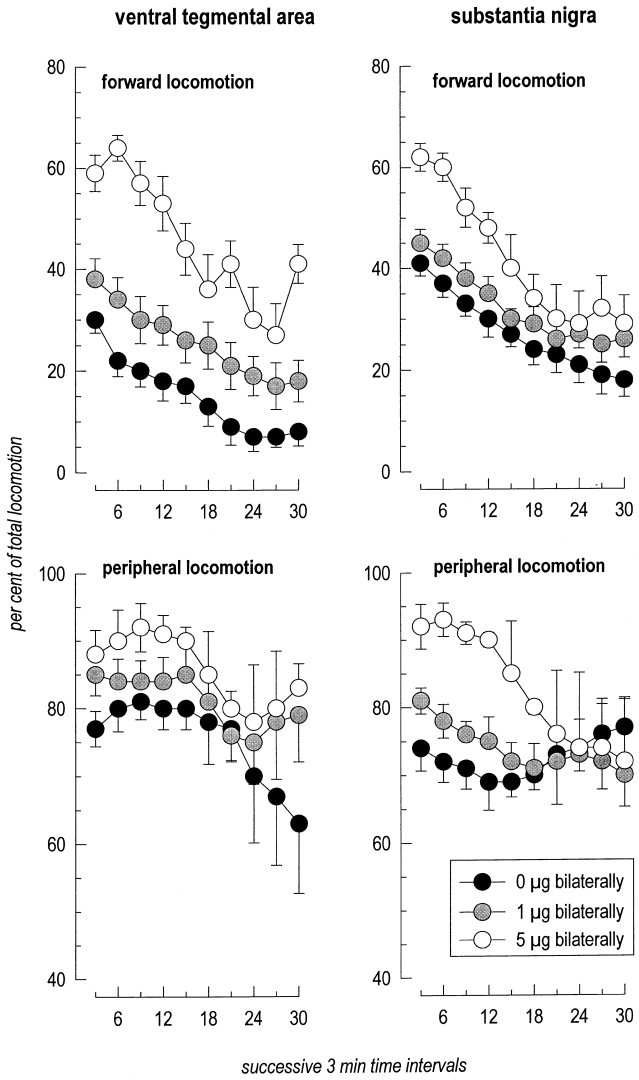

Fig. 3. (B) Effects of bilateral application of the 5-HT_{1A} receptor agonist 8-OH-DPAT into the ventral tegmental area (VTA), or the substantia nigra (SN), on forward locomotion and peripheral locomotion in the male rat. Forward locomotion (VTA), $F(2, 28)=30.99$, $p<0.01$ (dose); $F(9, 126)=20.68$, $p<0.01$ (time); $F(18, 252)=1.41$, $p>0.05$ (dose × time). Peripheral locomotion (VTA), $F(2, 28)=4.47$, $p<0.05$ (dose); $F(9, 126)=2.32$, $p<0.05$ (time); $F(18, 252)=0.56$, $p>0.01$ (dose × time). Forward locomotion (SN), $F(2, 24)=10.89$, $p<0.01$ (dose); $F(9, 108)=23.57$, $p<0.01$ (time); $F(18, 216)=2.57$, $p<0.01$ (dose × time). Peripheral locomotion (SN), $F(2, 24)=8.24$, $p<0.05$ (dose); $F(9, 108)=2.10$, $p<0.05$ (time); $F(18, 216)=1.60$, $p>0.05$ (dose × time).

sign [23]. Thus, rat #1 received the treatment sequence abc; rat #2 bca; rat #3 cab; etc. The doses of 8-OH-DPAT (0, 1, and 5 µg) were given weekly for 3 consecutive weeks. These behavioral experiments were analyzed by means of an appropriate, within-subjects repeated-measures, two-way ANOVA (A × B × S design) [20]. In the behavioral experiments, untreated controls ($n=5$) were run in parallel in the respective experiment. A mean value, based on the median from three open-field sessions was used in the statistical comparison with controls injected with saline into the VTA or the SN. The statistical comparison was performed by means of a classic two-way ANOVA for repeated measurements [20]. Because there were no statistically significant differences between the untreated controls run in parallel with VTA or SN experiments, these control groups were pooled in the presentation shown in

Fig. 2. The biochemical experiments were analyzed by means of a one-way ANOVA, followed by Dunnett's t-test for post hoc comparisons with appropriate controls. The scoring for presence of flat body posture was analyzed by means of the McNemar χ^2 test [30].

3. Results

3.1. Spontaneous open-field motor activity

3.1.1. Effects of local saline injections into the VTA or the SN on spontaneous open-field motor activity

As shown in Fig. 2, the open-field motor activity was affected by saline injections (0.5 μl per side) into either the VTA or the SN. Thus, there was a statistically significant increase in locomotion and rearing after a saline injection into the SN, $F(1, 16) = 12.94$, $p < 0.01$, and $F(1, 16) = 8.39$, $p < 0.02$, respectively). Furthermore, rearing, but not locomotion, was statistically significantly decreased after the saline injection into the VTA, $F(1, 18) = 5.44$, $p < 0.05$, and $F(1, 18) = 2.28$, $p > 0.05$, respectively. Finally, there was a highly significant habituation of locomotion and rearing, regardless of treatment, but there were no statistically significant interactions between treatment and time after injection.

3.1.2. Effects of 8-OH-DPAT on spontaneous open-field locomotor activity and rearing after local application into the VTA

There were marked, and statistically significant, effects of 8-OH-DPAT on locomotion and on rearing (Fig. 3A). Thus, 8-OH-DPAT stimulated locomotion, but the effect was maximal at the 1-μg dose, and no further increase was seen at 5 μg. The effects on rearing were more complex, with a decrease initially, followed by an increase with time. This pattern of effects was substantiated by a statistically significant interaction between dose and time (Fig. 3A).

Gross observations indicated a statistically significant display of flat body posture in 93% of animals treated with the highest dose of 8-OH-DPAT ($\chi^2 = 11.08$, $p < 0.01$). The percentage displaying a flat body posture was 7, 20, and 93% in animals given 0, 1, and 5 μg per side, respectively.

3.1.3. Effects of 8-OH-DPAT on spontaneous open-field locomotor activity and rearing after local application into the SN

There was a statistically significant suppression of both locomotion and rearing after the local application of 8-OH-DPAT into the SN (Fig. 3A). These effects were dose dependent, and particularly strong with regards to rearing activity. In addition, the statistical analysis indicated a statistically significant interaction between dose and time for both locomotion and rearing. Regarding locomotion, this appears due to a more rapid habituation in animals treated with the highest dose of 8-OH-DPAT. With regard to rearing, there was a marked suppression of activity, and this effect displayed very little change with time.

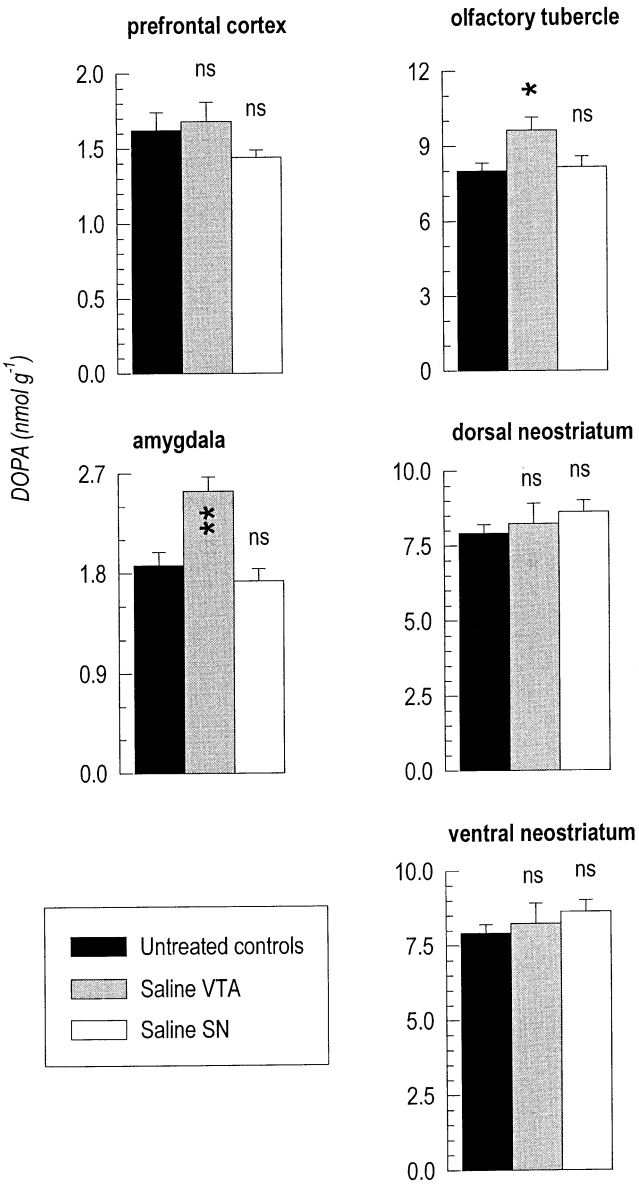

Fig. 4. Effects of saline control injections into the ventral tegmental area (VTA) or the substantia nigra (SN) on regional forebrain rate of catecholamine synthesis. The saline injection (0.5 μl side^{-1}) was given 5 min before the administration of NSD-1015 (100 mg kg^{-1}, IP). The animals were sacrificed 30 min following the NSD-1015 injection for subsequent biochemical analysis of regional brain DOPA accumulation. The figure shows means ± SEM based on 8–19 determinations per group. The results were statistically analysed by means of a one-way ANOVA, followed by the Dunnett's t-test for post hoc comparisons with untreated controls. Prefrontal cortex, $F(2, 36) = 0.67$, $p > 0.05$; amygdala, $F(2, 35) = 9.45$, $p < 0.01$; olfactory tubercle, $F(2, 35) = 4.41$, $p < 0.05$; dorsal neostriatum, $F(2, 36) = 0.62$, $p > 0.05$; ventral neostriatum, $F(2, 36) = 2.48$, $p > 0.05$. $^{ns}p > 0.05$; * $p < 0.05$; ** $p < 0.01$.

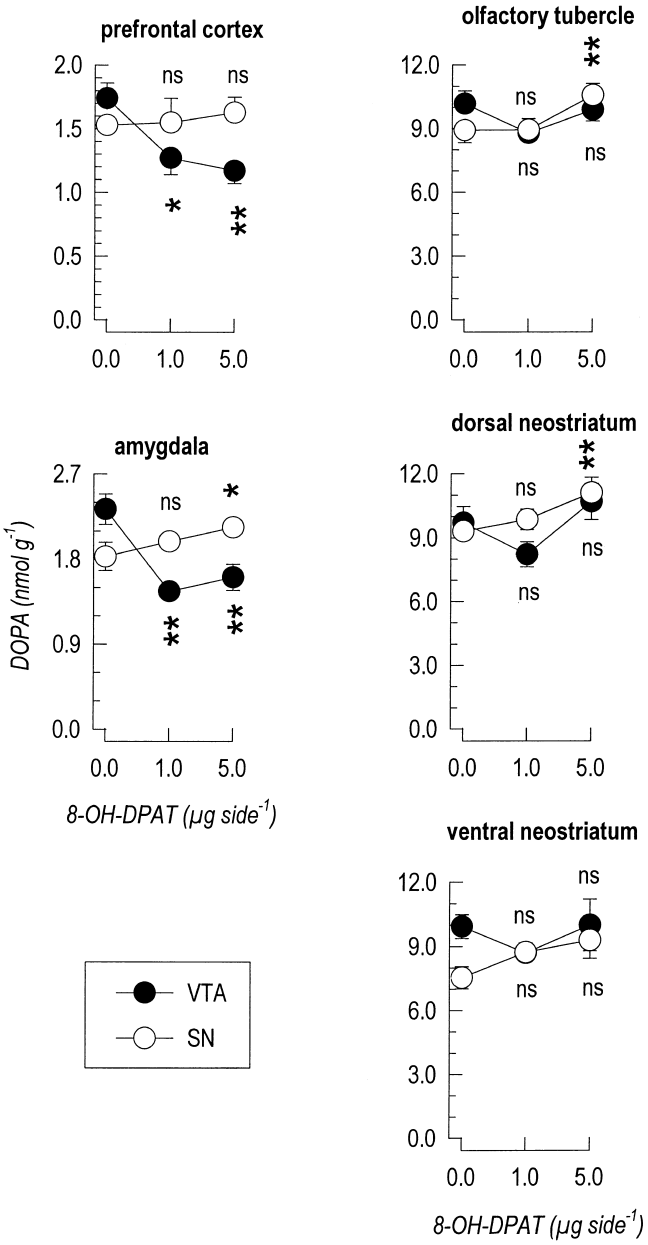

Fig. 5. Effects of bilateral injections of the 5-HT_{1A} receptor agonist 8-OH-DPAT into the ventral tegmental area (VTA), or the substantia nigra (SN), on regional forebrain catecholamine synthesis. The rats were injected with 8-OH-DPAT, and the DOPA decarboxylase inhibitor NSD-1015, 35 and 30 min, respectively, before being sacrificed for brain dissections and subsequent biochemical analysis of regional DOPA accumulation. The figure shows means±SEM based on three to five determinations per group. Statistical analysis was performed by means of separate one-way ANOVA's for VTA and SN, respectively, followed by post hoc comparisons with saline injected controls as indicated in the figure. VTA, $F(2, 12)=6.93$, $p<0.01$ (prefrontal cortex); $F(2, 12)=12.93$, $p<0.01$ (amygdala); $F(2, 12)=1.02$, $p>0.05$ (olfactory tubercle); $F(2, 12)=0.59$, $p>0.05$ (dorsal neostriatum); $F(2, 12)=0.49$, $p>0.05$ (ventral neostriatum). SN, $F(2, 14)=0.98$, $p>0.05$ (prefrontal cortex); $F(2, 14)=4.58$, $p<0.05$ (amygdala); $F(2, 14)=6.70$, $p<0.01$ (olfactory tubercle); $F(2, 14)=6.89$, $p<0.01$ (dorsal neostriatum); $F(2, 14)=1.63$, $p>0.05$ (ventral neostriatum).

There was a statistically significant display of flat body posture (92%) in animals given the 5-μg dose of 8-OH-DPAT ($\chi^2=10.08$, $p<0.01$), whereas no flat body posture was observed in animals given the lower dose of 8-OH-DPAT, or in saline treated controls (0% in both cases).

3.1.4. Effects of 8-OH-DPAT on spontaneous open-field forward and peripheral locomotion after local application into the VTA and SN

The proportion forward and peripheral locomotion increased in a dose-dependent manner after administration of 8-OH-DPAT into both the VTA and the SN (Fig. 3B). The effect was clearly time dependent and, except for effects of 8-OH-DPAT on forward locomotion after local application into the SN, there were no statistically significant interactions between dose and time after injection (Fig. 3B).

3.2. Forebrain catecholamine rate of synthesis

3.2.1. Effects of local saline injections into the VTA or the SN on forebrain DOPA accumulation

The 0.5 μl saline injection into the VTA, but not into the SN, produced a statistically significant increase in the DOPA accumulation in the amygdala and the olfactory tubercle, in comparison with untreated controls (Fig. 4). There were no effects of the saline injection into the VTA, or the SN, on DOPA accumulation in other brain areas.

3.2.2. Effects of 8-OH-DPAT on forebrain DOPA accumulation after local application into the VTA

There was a statistically significant decrease in the DOPA accumulation in the prefrontal cortex and in the amygdala, but not in the neostriatal brain areas, after 8-OH-DPAT injections into the VTA (Fig. 5). The effect appeared to be maximal already at the 1-μg dose of 8-OH-DPAT.

3.2.3. Effects of 8-OH-DPAT on forebrain DOPA accumulation after local application into the SN

At the highest dose of 8-OH-DPAT, there was a statistically significant increase in the DOPA accumulation in the amygdala, the olfactory tubercle and in the dorsal neostriatum (Fig. 5). The DOPA accumulation in the prefrontal cortex or in the ventral neostriatum were not affected, nor were there any statistically significant effects by the 1-μg dose in any brain area.

4. Discussion

The local application of 8-OH-DPAT into either the VTA or the SN produced distinct changes in the pattern of spontaneous locomotor activity within the open field. To some extent, these changes were similar to the effects seen by systemic 8-OH-DPAT administration [2,5,13,15]. First, the intranigral 8-OH-DPAT application resulted in a pattern of activity similar, albeit less pronounced, to that

seen after systemic 8-OH-DPAT, i.e., suppression of total locomotion and rearing, as well as an increase in the proportion of forward and peripheral locomotion. Second, the local application into the VTA produced a more uniform pattern of behavioral activation, i.e., total locomotion, as well as forward locomotion, were increased. However, the decrease and increase in rearing and peripheral locomotion were less conspicuous than after injections into the SN.

Evidence in support of DA receptor agonistic effects of 8-OH-DPAT has been obtained in biochemical and electrophysiological, as well as in physiological preparations. First, systemic administration of 8-OH-DPAT to reserpine-pretreated rats results in a clear, and raclopride-sensitive, decrease in limbic forebrain DA rate of synthesis [3]. In normal rats, however, there is a modest increase in DA turnover [4]. It is well known that partial DA D_2 receptor agonists may have agonist actions at supersensitive DA receptors, produced by reserpine, for example, and antagonist actions at normosensitive receptors. The partial DA D_2 receptor agonist (−)-3-(3-hydroxypenyl)-*N*-*n*-propyl-piperidine (3-PPP) is a case in point (see, e.g., [16]). In further agreement with the notion of intrinsic efficacy of 8-OH-DPAT at brain DA receptors, DA release in the SN has been shown to decrease after systemic 8-OH-DPAT administration, and this effect was not sensitive to pretreatment with pindolol [26]. Second, 8-OH-DPAT produces a haloperidol-sensitive reduction of firing in nigrostriatal DA neurons in anaesthetized rats [31]. A similar, raclopride-sensitive, effect is seen with (+)-8-OH-DPAT in both SN and VTA, albeit at higher doses only [7]. It is probable, however, that racemic 8-OH-DPAT has stronger dopaminergic effects than the (+)enantiomer because most of such effects appear to reside in the (−)enantiomer of 8-OH-DPAT [22]. Third, in a vas deferens preparation, 8-OH-DPAT behaves as a partial DA receptor agonist [32]. Thus, the 8-OH-DPAT-induced effect was completely antagonized by the addition of DA D_2 receptor antagonists, but the maximal effect of 8-OH-DPAT by itself was considerably less than the maximal effect obtained by apomorphine.

It was recently reported that 8-OH-DPAT, as well as 5-HT, display intrinsic efficacy in a CHO cell line preparation transfected with DA D_2 receptors [28]. Although this finding supports a direct effect of 8-OH-DPAT at DA D_2 receptors, it should be noted that an estimated intrinisic efficacy of about 50% for 8-OH-DPAT or 5-HT at DA D_2 receptors appears somewhat optimistic for the in vivo situation. Thus, for example, 5-HTP treatment is not a viable alternative to partial DA D_2 receptor agonists in the treatment of Parkinson's disease (cf. Ref. [18]).

From the above, it appears that the effects produced by 8-OH-DPAT, at least partially, are mediated via DA D_2 receptors in the SN and the VTA. Considering the weak intrinsic efficacy displayed by 8-OH-DPAT (cf. Ref. [6]), it is probable that DA D_2 receptor antagonism is the predominant effect in a normal awake animal. In support of this interpretation, the DA rate of synthesis, estimated as the DOPA accumulation following inhibition of cerebral decarboxylase, was increased in neostriatal projection terminal areas after the intranigral application. After the local application into the VTA, however, a different pattern of effects emerged. Thus, neostriatal DA synthesis was not significantly affected, whereas the mesocortical projection reacted in a very specific manner. Thus, there was a clear and statistically significant decrease in the DOPA accumulation in the prefrontal cortex, and the amygdala, after the application of 8-OH-DPAT into the VTA. These findings suggest an inhibitory role of the mesocortical projection at the behavioral level. The explanation for this VTA-specific effect could be differential effects of 8-OH-DPAT on different subpopulations of DA neurons in the VTA (cf. Ref. [21]). It should also be noted that, although 5-HT_{1A} receptors have not been demonstrated in the VTA, there is a weak signal for 5-HT_{1A} receptor mRNA in this area [34], suggesting an additional explanation for the very selective effects of 8-OH-DPAT injections into the VTA on mesocortical dopaminergic synthesis.

Two important methodological issues in the present type of study are (1) effects of the injection procedure on the animals, and (2) escape of the injected materials into systemic circulation. In regard to the first issue, injections of the saline vehicle into either the SN or the VTA affected behavior, as well as forebrain DA rate of synthesis. The two sites reacted differently, however, to the vehicle injection. Thus, a modest behavioral suppression of the spontaneous locomotor activity was seen after the VTA injection, whereas a corresponding stimulation of activity was seen after the intranigral injection. Furthermore, certain areas innervated by mesocortico-limbic projections, i.e., the amygdala and olfactory tubercle, as dissected here, reacted with an increased rate of DA synthesis as a result of the application of vehicle into the VTA, whereas no effects were noted after the intranigral injection.

Diffusion of 8-OH-DPAT and escape into systemic circulation is an important concern after intracerebral application of the compound [1]. Thus, with time, 8-OH-DPAT in all probability will have effects outside the intended site of injection. It should be noted, however, that within the time window used here, 8-OH-DPAT exerted different behavioral and biochemical effects after its local application into the SN or the VTA, excluding a predominance of effects due to systemic distribution of 8-OH-DPAT.

5. Conclusion

The local application of 8-OH-DPAT into the SN or the VTA produced behavioral stimulation, as expressed by a substantial increase in the proportion forward locomotion,

relative to total locomotion. After the intranigral injections, this pattern of activity was similar to the stereotyped forward locomotion previously reported to occur after systemic 8-OH-DPAT administration, whereas the VTA injection produced a more general activation of the animals. The effects of the SN or the VTA injections on forebrain DA synthesis were distinctly different. Thus, 8-OH-DPAT behaved as an antagonist on DA D_2 autoreceptors in the SN, whereas the inhibitory effects on DA synthesis in the mesocortical projection indicated an agonist action of 8-OH-DPAT on dopaminergic autoreceptors in the VTA. The net effect of systemically administered 8-OH-DPAT will depend on pre- and postsynaptic effects on serotonergic as well as dopaminergic brain mechanisms. The present results demonstrate that mesencephalic DA autoreceptors is an important target for 8-OH-DPAT.

Acknowledgments

For expert and unfailing help, we would like to thank Mr. Thomas Andersson and his crew at the Animal Department.

References

[1] Ahlenius S, Briem S, Hillegaart V, Magnusson O. Diffusion and absorption of (−) sulpiride and raclopride after intracerebral administration in the rat. Biochem Pharmacol 1990;39:2066–9.

[2] Ahlenius S, Hillegaart V, Salmi P, Wijkström A. Effects of 5-HT_{1A} receptor agonists on patterns of rat motor activity in relation to effects on forebrain monoamine synthesis. Pharmacol Toxicol 1993;72:398–406.

[3] Ahlenius S, Hillegaart V, Wijkström A. Evidence for selective inhibition of limbic forebrain dopamine synthesis by 8-OH-DPAT in the rat. Naunyn-Schmiedeberg's Arch Pharmacol 1989;339:551–6.

[4] Ahlenius S, Hillegaart V, Wijkström A. Increased dopamine turnover in the ventral striatum by 8-OH-DPAT administration in the rat. J Pharm Pharmacol 1990;42:285–8.

[5] Ahlenius S, Larsson K, Wijkström A. Behavioral and biochemical effects of the 5-HT_{1A} receptor agonists flesinoxan and 8-OH-DPAT in the rat. Eur J Pharmacol 1991;200:259–66.

[6] Ahlenius S, Salmi P. Antagonism of reserpine-induced suppression of spontaneous motor activity by stimulation of 5-HT_{1A} receptors in rats. Pharmacol Toxicol 1995;76:149–56.

[7] Arborelius L, Chergui K, Murase S, Nomikos GG, Backlund Höök B, Chouvet G, Hacksell U, Svensson TH. The 5-HT_{1A} receptor ligands, (*R*)-8-OH-DPAT and (*S*)-UH-301, differentially affect the activity of midbrain dopamine neurons. Naunyn-Schmiedeberg's Arch Pharmacol 1993;347:352–62.

[8] Arvidsson L-E, Hacksell U, Nilsson JLG, Hjorth S, Carlsson A, Lindberg P, Sanchez D, Wikström H. 8-Hydroxy-2-(di-*n*-propylamino)tetralin, a new centrally acting 5-hydroxytryptamine receptor agonist. J Med Chem 1981;24:921–3.

[9] Carlsson A, Davis JN, Kehr W, Lindqvist M, Atack CV. Simultaneous measurement of tyrosine and tryptophan hydroxylase activities in brain in vivo using an inhibitor of the aromatic amino acid decarboxylase. Naunyn-Schmiedeberg's Arch Pharmacol 1972;275:153–68.

[10] Ericson E, Samuelsson J, Ahlenius S. Photocell measurements of rat motor activity. J Pharmacol Methods 1991;25:111–22.

[11] Hadrava V, Blier P, Dennis T, Ortemann C, de Montigny C. Characterization of 5-hydroxytryptamine$_{1A}$ properties of flesinoxan: in vivo electrophysiology and hypothermia study. Neuropharmacology 1995;34:1311–26.

[12] Hillegaart V. Effects of local application of 5-HT and 8-OH-DPAT into the dorsal and median raphe nuclei on motor activity in the rat. Physiol Behav 1990;48:143–8.

[13] Hillegaart V, Estival A, Ahlenius S. Evidence for specific involvement of 5-HT_{1A} and 5-$HT_{2A/C}$ receptors in the expression of patterns of spontaneous motor activity of the rat. Eur J Pharmacol 1996; 295:155–61.

[14] Hillegaart V, Hjorth S, Ahlenius S. Effects on forebrain monoamine synthesis by the local application of 5-HT and 8-OH-DPAT into the median and dorsal raphe nuclei of the rat. J Neural Transm 1990;81:131–45.

[15] Hillegaart V, Wadenberg M-L, Ahlenius S. Effects of 8-OH-DPAT on motor activity in the rat. Pharmacol Biochem Behav 1989; 32:797–800.

[16] Hjorth S, Carlsson A, Clark D, Svensson K, Wikström H, Sanchez D, Lindberg P, Hacksell U, Arvidsson L-E, Johansson A, Nilsson JLG. Central dopamine receptor agonist and antagonist actions of the enantiomers of 3-PPP. Psychopharmacology (Berlin) 1983;81:89–99.

[17] Hjorth S, Carlsson A, Lindberg P, Sanchez D, Wikström H, Arvidsson L-E, Hacksell U, Nilsson JLG. 8-Hydroxy-2-(di-*n*-propylamino)-tetralin, 8-OH-DPAT, a potent and selective simplified ergot congener with central 5-HT-receptor stimulating activity. J Neural Transm 1982;55:169–88.

[18] Jankovic J, Marsden CD. Therapeutic strategies in Parkinson's Disease. In: Jankovic J, Tolosa E, editors. Parkinson's Disease and movement disorders. Baltimore: Williams and Wilkins, 1998. pp. 191–220.

[19] Kalkman HO, Soar J. Determination of the 5-HT receptor subytpe involved in 8-OH-DPAT-induced hyperlocomotion — potential difficulties arising from inadequate pharmacological tools. Eur J Pharmacol 1990;191:383–90.

[20] Keppel G. Design and analysis. A researcher's handbook. Englewood Cliffs, NJ: Prentice-Hall, 1973.

[21] Kiyatkin EA, Rebec GV. Heterogeneity of ventral tegmental area neurons: Single-unit recording and iontophoresis in awake, unrestrained rats. Neuroscience 1998;85:1285–309.

[22] Lejeune F, Newman-Tancredi A, Audinot V, Millan MJ. Interactions of (+)- and (−)-8- and 7-hydroxy-2-(di-*n*-propylamino)tetralin at human (h)D_3, hD_2 and h serotonin$_{1A}$ receptors and their modulation of the activity of serotoninergic and dopaminergic neurones in rats. J Pharmacol Exp Ther 1997;280:1241–9.

[23] Li CC. Introduction to experimental statistics. New York: McGraw-Hill, 1964.

[24] Magnusson O, Nilsson LB, Westerlund D. Simultaneous determination of dopamine, DOPAC and homovanillic acid. Direct injections of supernatants from brain tissue homogenates in a liquid chromatography — electrochemical detection system. J Chromatogr 1980; 221:237–47.

[25] Mittman SM, Geyer MA. Effects of 5-HT_{1A} agonists on locomotor and investigatory behaviors in rats differ from those of hallucinogens. Psychopharmacology (Berlin) 1989;98:321–9.

[26] Nissbrandt H, Waters N, Hjorth S. The influence of serotoninergic drugs on dopaminergic neurotransmission in rat substantia nigra, striatum and limbic forebrain in vivo. Naunyn-Schmiedeberg's Arch Pharmacol 1992;346:12–9.

[27] Paxinos G, Watson C. The brain in stereotaxic coordinates. San Diego: Academic Press, 1998.

[28] Rinken A, Ferré S, Terasmaa A, Owman C, Fuxe K. Serotonergic agonists behave as partial agonists at the dopamine D_2 receptor. Neuro Report 1999;10:493–5.

[29] Schipper J, Tulp MThM, Berkelmans B, Mos J, Van der Heijden B, Olivier B. Preclinical pharmacology of flesinoxan: a potential anxiolytic and antidepressant drug. Hum Psychopharmacol 1991; 6:S53–S61.

[30] Siegel S. Nonparametric statistics for the behavioral sciences. New York: McGraw-Hill, 1956.

[31] Sinton CM, Fallon SL. Electrophysiological evidence for a functional differentiation between subtypes of the 5-HT_1 receptor. Eur J Pharmacol 1988;157:173–81.
[32] Smith CFC, Cutts S. Dopamine agonist activity of 8-OH-DPAT. Arch Int Pharmacodyn Ther 1990;306:106–13.
[33] Tricklebank MD, Forler C, Fozard JR. The involvement of subtypes of the 5-HT_1 receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-*n*-propylamino)tetralin in the rat. Eur J Pharmacol 1984;106:271–82.
[34] Wright DE, Seroogy KB, Lundgren KH, Davis BM, Jennes L. Comparative localization of serotonin$_{1A,\ 1C}$, and $_2$ receptor subtype mRNAs in rat brain. J Comp Neurol 1995;351:357–73.