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Involvement of dopamine D_2 receptors in apomorphine-induced facilitation of forebrain serotonin output

Anna Mendlin *, Francisco J. Martín, Barry L. Jacobs

Program in Neuroscience, Green Hall, Princeton University, Princeton, NJ 08544-1010, USA

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Abstract

The effect of systemic administration of the nonselective dopamine receptor agonist apomorphine on efflux of serotonin (5-hydroxytryptamine, 5-HT) in striatum and hippocampus of freely moving rats was examined using in vivo microdialysis. 5-HT efflux was increased by a moderate dose of apomorphine sufficient for a postsynaptic dopaminergic effect (0.5 mg/kg, s.c.), but not by a lower dose (0.1 mg/kg, s.c.), that acts preferentially on presynaptic dopamine receptors. This effect was blocked by a dopamine D_2 receptor antagonist raclopride, administered either systemically or locally into striatum, but not by a 5-HT_{1A} receptor antagonist *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl) cyclohexanecarboxamide 3HCl (WAY-100635). This indicates that dopamine D_2 receptors, and not 5-HT_{1A} receptors, mediate the facilitatory effect of apomorphine, and that this effect occurs at the nerve terminal level. Behavioral effects of apomorphine outlasted the concomitant changes in 5-HT efflux, suggesting that these changes resulted from dopaminergic receptor activation, rather than from the drug-induced behavioral arousal.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); 5-HT_{1A} autoreceptor; Dopamine; Dopamine D_2 receptor; Stereotypy; Microdialysis

1. Introduction

An extensive body of neuroanatomical, electrophysiological and biochemical data supports the existence of a functional interaction between central serotonin (5-hydroxytryptamine, 5-HT) and dopamine. Both basic and clinical research provide evidence for the relevance of this interaction to the etiology and treatment of mental disorders such as schizophrenia (Meltzer and Nash, 1991; Abi-Dargham et al., 1997) and depression (Mann and Kapur, 1995; Willner, 1995).

Although a large number of experiments have been directed at the effects of serotonin on the dopaminergic system, the effects of dopamine on the 5-HT system have not been studied extensively. Anatomical studies demonstrate that a morphological basis exists for dopaminergic modulation of the 5-HT system, either at the level of serotonergic cell bodies, or at the level of the nerve terminals. Retrograde labeling studies (Kalen et al., 1988; Peyron et al., 1996) demonstrated that dopaminergic neurons from the ventral tegmental area give rise to a terminal plexus in the dorsal raphe nucleus. Dopamine-containing neurons were observed in the raphe nuclei, where the dopaminergic fibers make contact with indoleaminergic cell bodies and fibers (Geffard et al., 1987; Kalen et al., 1988). Moderate levels of dopamine D_2 receptor mRNA, as well as dopamine D_1 and D_2 receptor binding were reported in the raphe nuclei (Mansour et al., 1990). Dopaminergic and serotonergic axon terminals also converge in such forebrain areas as the basal ganglia and portions of the limbic system (Le Moal and Simon, 1991; Jacobs and Azmitia, 1992). Studies using either synaptosomes or hippocampal slice preparations suggested the existence of facilitatory presynaptic dopamine receptors located on serotonergic nerve terminals (Balfour and Iyaniwura, 1985). Both dopamine D_1 and D_2 receptor subtypes are present at medium to high densities throughout the basal ganglia and the limbic system (Bouthenet et al., 1987; Jackson and Westlind-Danielsson, 1994).

Several studies demonstrated that enhancing dopaminergic transmission via systemic administration of moderate doses of amphetamine, methylphenidate or apomorphine selectively increases utilization and/or tissue levels of 5-HT in the areas innervated by the dorsal raphe nucleus,

* Corresponding author. Tel.: +1-609-258-6606; fax: +1-609-258-1113; e-mail: annam@princeton.edu

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such as the corpus striatum (Grabowska et al., 1973; Lee and Geyer, 1984; Lee, 1987). Reducing dopamine content in the corpus striatum by chronic blockade of dopamine reuptake resulted in increased 5-HT turnover, suggesting possible disinhibition of striatal 5-HT metabolism (Sivam, 1995). In vitro data are less consistent: [^{3}H]5-HT release from the forebrain was unaffected by stimulation of dopamine receptors in some studies (Baumann and Waldmeier, 1981; Feuerstein et al., 1986), and increased in others (Kelly et al., 1985; Balfour and Iyaniwura, 1985).

Characteristics of the dopaminergic modulation of 5-HT neurotransmission in vivo are unclear at present. Systemic administration of the nonselective dopamine receptor agonist apomorphine increases the firing rate of dorsal raphe 5-HT neurons in both cats (Fornal et al., 1996) and rats (Martín-Ruiz et al., 1997). Stimulation of dopamine receptors facilitates 5-HT outflow in several forebrain areas (Petty et al., 1994; Matsumoto et al., 1996; Martín-Ruiz et al., 1997), although a slight reduction of 5-HT efflux has also been reported in the corpus striatum after apomorphine administration (Ferré et al., 1994). Receptor mechanisms mediating effects of dopamine on 5-HT transmission are not well understood. Effects of systemic apomorphine administration on 5-HT output were blocked by the 5-HT$_{1A}$ receptor antagonist WAY-100135 (Ferré et al., 1994). Nevertheless, the apomorphine-induced increases in the firing rate of 5-HT neurons in the dorsal raphe, were potentiated, rather than prevented, by pretreatment with the more selective 5-HT$_{1A}$ receptor antagonist WAY-100635 (Fornal et al., 1996). Apomorphine effects on 5-HT transmission were mimicked by dopamine D$_2$, but not by D$_1$, receptor agonists and were blocked by D$_2$ receptor antagonists in the dorsal raphe (Ferré et al., 1994; Martín-Ruiz et al., 1997), as well as in the hippocampus (Matsumoto et al., 1996), suggesting that dopamine D$_2$ receptors are involved in mediating dopaminergic modulation of 5-HT transmission. This modulation might also be region-specific. The hippocampus and corpus striatum receive most of their serotonergic input from median and dorsal raphe nuclei, respectively (Jacobs and Azmitia, 1992). Dopamine D$_2$ receptor activation was shown to elevate the dialysate levels of 5-HT in the dorsal (Ferré and Artigas, 1993), but not in the median raphe nucleus (Adell and Artigas, 1997). The hippocampus and corpus striatum also have different sources of dopaminergic innervation and different dopamine receptor density (Bouthenet et al., 1987; Jackson and Westlind-Danielsson, 1994).

It is well documented that 5-HT efflux closely parallels general behavioral activation of the animal (Mendlin et al., 1996; Rueter and Jacobs, 1996; Portas et al., 1998). Since systemic administration of a dopamine receptor agonist, in doses sufficient to activate postsynaptic dopamine receptors, elicits behavioral stereotypies and increases locomotor activity in rodents (Costall et al., 1972), it is important to attempt to distinguish changes in 5-HT output resulting directly from dopaminergic stimulation from those that might occur due to the general behavioral activation produced by the drug.

The present study examined the effect of systemic apomorphine administration on 5-HT efflux in the corpus striatum and hippocampus of freely moving rats and assessed the roles of 5-HT$_{1A}$ receptors and dopamine D$_2$ receptors in mediating this effect. In addition, we addressed the question of whether the effect of systemic apomorphine administration was mediated by dopamine D$_2$ receptors in the corpus striatum. Finally, in order to determine whether the changes in 5-HT efflux were attributable to dopaminergic receptor stimulation, rather than to a concomitant behavioral state change, apomorphine-evoked stereotyped behavior and general behavioral arousal of the animals were monitored throughout the experiments.

2. Materials and methods

Male Sprague–Dawley rats weighing 220–260 g were housed individually under controlled temperature and lighting conditions (22 ± 0.5°C; 12-h reversed light/dark cycle, white light off/dim red light on at 11:00 a.m.) with food and water available ad libitum. All experimental protocols were approved by the Animal Care and Use Committee of Princeton University. For surgery, rats were pretreated with atropine HCl (0.2 mg/kg, i.p.), and anesthetized with a mixture of ketamine HCl and xylazine (80 mg/kg and 12 mg/kg, respectively, i.m.). Animals were placed in a stereotaxic frame in a flat-skull position and stainless-steel guide cannulae (9 mm length, 0.7 mm o.d.) were implanted in the corpus striatum (AP -0.5 mm, ML ± 3.0 mm from bregma, DV -2.6 mm below dura) and hippocampus (AP $+5.4$ mm, ML ± 4.8 mm from bregma, DV -2.2 mm below dura), according to the atlas of Paxinos and Watson (1986). The cannulae were secured with skull screws and dental acrylic and plugged with stainless-steel stylets. Postoperatively, rats received an injection of penicillin (300 000 U/kg, i.p) and were allowed to recover for 4–7 days. Concentric dialysis probes (4.0 mm length of nitrocellulose membrane, 0.22 mm o.d., 6000 Da cut-off; Spectrum, Houston, TX) were constructed as previously described (Hernandez et al., 1987). On the day of an experiment, rats were gently restrained without use of anesthesia and dialysis probes were lowered through the guide cannulae and secured with dental acrylic, so that the probe tips extended 4 mm beyond the cannulae tips. The probe inlets were attached to a Harvard syringe microinfusion pump (Harvard Apparatus, Boston, MA) via a fluid swivel, and a modified Ringers solution (147.2 mM NaCl, 4.0 mM KCl, 1.8 mM $CaCl_2$) was continuously infused at a flow rate of 1.3 μl/min. The perfusion medium contained 3 μM fluoxetine (Eli Lilly, Indianapolis, IN); this concentration has been shown to be selective for 5-HT without affecting the efflux of dopamine (Benloucif and Galloway, 1991; Li et al., 1996b).

A reversed phase high performance liquid chromatography system coupled with electrochemical detection (HPLC-ECD) was used for the analysis of dopamine, 3,4-dihydrophenylacetic acid (DOPAC), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA). The mobile phase (0.15 M chloroacetic acid, 0.12 M NaOH, 0.18 mM EDTA, 60 ml/l acetonitrile and 1.0 mM sodium octane sulfate) was delivered at a flow rate of 1.0 ml/min onto a 10 cm × 3.2 mm ODS 3 μm column (BAS, W. Lafayette, IN). Perfusate samples were collected at 20-min intervals, manually injected (model 7125 injector, Rheodyne, Cotati, CA) and analyzed using a dual potentiostat electrochemical detector (model 400 EG&G, Princeton Applied Research, Princeton, NJ), with the potentials applied to the parallel working electrodes set at 610 and 590 mV relative to an Ag/AgCl reference electrode. A Shimadzu model C-R3A integrator (Kyoto, Japan) was used to analyze the output from the detector. Identification and quantification of compounds in the samples was achieved by comparison of the retention times and peak heights to those of a standard solution containing dopamine, DOPAC, 5-HT and 5-HIAA. The detection limit for 5-HT was approximately 1 pg based on a signal-to-noise ratio of 3:1. In vitro probe recovery was determined by immersing the probes in a standard solution containing 10 pg of each dopamine and 5-HT and perfusing them for at least 4 h with a Ringers solution at a flow rate of 1.3 μl/min. The relative recovery was 13.2 ± 3.8% for 5-HT and 12.1 ± 3.7% for dopamine (means ± S.E.M., $n = 8$).

Sample collection started 3 h after the probe implantation. Immediately after obtaining a stable four-sample baseline, apomorphine HCl (Research Biochemicals International, Natick, MA) was administered (0.5 mg/kg, s.c.), and five more samples were collected. Immediately after that, one of the following pretreatments was administered: the serotonin 5-HT_{1A} receptor antagonist WAY-100635 (*N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl) cyclohexanecarboxamide 3HCl, Wyeth-Ayerst, Princeton, NJ; 0.1 mg/kg, s.c.); the dopamine D_2 receptor antagonist raclopride (Research Biochemicals International; 0.5 mg/kg, s.c. or 10 μM added to striatal perfusate for 2 h); or saline (1 ml/kg, s.c.). After collecting one more dialysate sample, the apomorphine injection was repeated and four additional samples were collected. In a series of control experiments, the animals received one of the following treatments: injection of apomorphine (0.1 mg/kg, s.c.) or raclopride (0.5 mg/kg, s.c.), or application of 10 μM raclopride through the striatal dialysis probe; follow-up dialysate samples were collected for 2 h after each of the manipulations.

Animals were videotaped for the duration of the experiments, and two types of scores—stereotypy ratings and general behavioral activation ratings—were assigned later in a blind fashion for each sampling period. Ratings for the general behavioral activation were assigned for each 20-min period by determining the amount of time spent in active waking (gross bodily movements or maintained postural support). Stereotypy ratings were determined for all sampling periods following the first injection of apomorphine. The stereotypies were scored according to Costall et al. (1972): 0—no stereotypy; 1—discontinuous sniffing and/or repetitive head and limb movements; 2—continuous sniffing and/or repetitive head and limb movements; 3—periodic chewing, biting or licking; 4—continuous chewing, biting or licking. In order to compare the changes in 5-HT output with the concomitant stereotyped behavior, the intensity of the stereotypy was determined from three 30-s samples obtained at the beginning, middle and end of each 20-min interval, yielding a cumulative score from 0 to 12.

For verification of probe placement, animals were perfused intracardially with 10% formalin in saline under deep phenobarbital anesthesia (100 mg/kg, i.p.). Brains were removed, serial frozen sections (75 μm thick) were cut using a microtome, mounted on glass slides and stained with neutral red. The slides were examined under a microscope and the data from the animals with incorrect probe placement were discarded.

To minimize between-subject variation, levels of all compounds were expressed as a percentage of the mean of four baseline samples. For each of the two apomorphine injections, the area under the curve of 5-HT was calculated for four consecutive samples, starting immediately after each apomorphine treatment. All values are expressed as means ± S.E.M (n equals the number of animals included in each analysis). All data were analyzed using a two-way analysis of variance (ANOVA) (mixed design with repeated-measures analysis with time as a within-subject factor) followed by post hoc comparisons (Student–Newman–Keuls' test).

3. Results

As shown in Table 1 and Fig. 1, apomorphine administration (0.5 mg/kg, s.c.) increased 5-HT concentration to 179 ± 11% of baseline in the corpus striatum and 227 ± 14% of baseline in hippocampus during the first sample ($F(13,221) = 34.1$, $P < 0.01$, $n = 21$, and $F(13, 208) = 66.1$, $P < 0.01$, $n = 19$, respectively). 5-HT output returned to pre-drug levels within two sampling periods (see Fig. 1). Apomorphine also significantly reduced the efflux of dopamine in the corpus striatum ($F(13,156) = 11.6$, $P < 0.01$, $n = 16$) and efflux of DOPAC both in the corpus striatum and hippocampus ($F(13,208) = 35.9$, $P < 0.01$, $n = 20$ and $F(13,195) = 6.3$, $P < 0.01$, $n = 19$, respectively). Administration of a lower dose of apomorphine (0.1 mg/kg, s.c.) had no effect on 5-HT output in either area ($F(10,50) = 0.7$, $P = 0.75$, $n = 6$ and $F(10,50) = 0.8$, $P = 0.62$, $n = 6$, respectively); this dose significantly reduced the efflux of dopamine in the corpus striatum ($F(10,50) = 11.3$, $P < 0.01$, $n = 6$) and efflux of DOPAC

Table 1
Maximal effects produced by apomorphine with or without pretreatments on the output of 5-HT, dopamine and their metabolites in the corpus striatum and hippocampus

	Apo 0.1	Apo 0.5	Apo + Sal	Apo + WAY	Apo + SRac	SRac	LRac	Apo + LRac
Corpus striatum								
5-HT	106.0 ± 8.3	179.4 ± 11.3*	184.0 ± 14.8*	203.3 ± 16.9*	97.2 ± 9.2	89.3 ± 7.6	104.6 ± 7.4	126.2 ± 10.3
5-HIAA	97.6 ± 6.3	103.2 ± 2.3	97.3 ± 2.7	102.7 ± 2.6	107.4 ± 3.0	105.4 ± 2.5	96.1 ± 4.9	94.0 ± 3.3
Dopamine	60.2 ± 9.0*	37.5 ± 8.1*	20.7 ± 6.8*	35.0 ± 5.6*	88.0 ± 6.5	149.2 ± 6.6*	196.0 ± 12.8*	94.0 ± 3.9
DOPAC	51.1 ± 3.8*	32.8 ± 6.3*	35.5 ± 4.3*	37.1 ± 3.7*	121.7 ± 5.3	240.1 ± 6.9*	131.0 ± 5.3*	86.0 ± 12.1
Hippocampus								
5-HT	94.3 ± 9.1	227.0 ± 14.3*	244.0 ± 10.8*	236.0 ± 7.6*	107.0 ± 9.4	109.8 ± 6.7		
5-HIAA	103.2 ± 4.0	96.3 ± 5.8	113.0 ± 5.0	105.2 ± 3.0	112.0 ± 2.8	95.0 ± 4.1		
DOPAC	61.0 ± 3.9*	32.0 ± 5.7*	35.0 ± 4.1*	58.0 ± 6.5*	92.4 ± 4.8	116.2 ± 9.5		

All pretreatments were administered 20 min prior to apomorphine (0.5 mg/kg, s.c.). Abbreviations: apomorphine, 0.1 mg/kg, s.c. (Apo 0.1); apomorphine, 0.5 mg/kg, s.c. (Apo 0.5); saline pretreatment, 0.1 ml/kg (Apo + Sal); WAY-100635 pretreatment, 0.1 mg/kg (Apo + WAY); systemic raclopride pretreatment, 0.5 mg/kg (Apo + SRac); local raclopride pretreatment, 10 μM added to striatal perfusate (Apo + LRac); local raclopride alone, 10 μM added to striatal perfusate for 2 h (LRac); systemic raclopride alone, 0.5 ml/kg (SRac). Each cell represents mean values ± S.E.M. of five to seven animals. Data are expressed as percentages of four baseline samples. * $P < 0.05$ compared to baseline; Student–Newman–Keuls' multiple comparisons test. In the presence of 3 μM fluoxetine the basal levels of 5-HT amounted to 0.18 ± 0.09 pg/μl in corpus striatum and 0.19 ± 0.06 pg/μl in hippocampus; levels of dopamine were 0.37 ± 0.12 pg/μl in corpus striatum and below detection limit in hippocampus; levels of 5-HIAA equaled to 3.28 ± 0.3.0 ng/μl in corpus striatum and 4.78 ± 0.57 ng/μl in hippocampus; levels of DOPAC were 7.5.0 ± 0.75 ng/μl in corpus striatum and 3.8.0 ± 0.82 ng/μl in hippocampus ($n = 7$ for all groups).

both in the corpus striatum and hippocampus ($F(10,50) = 12.4$, $P < 0.01$, $n = 6$ and $F(10,50) = 6.4$, $P < 0.01$, $n = 6$, respectively).

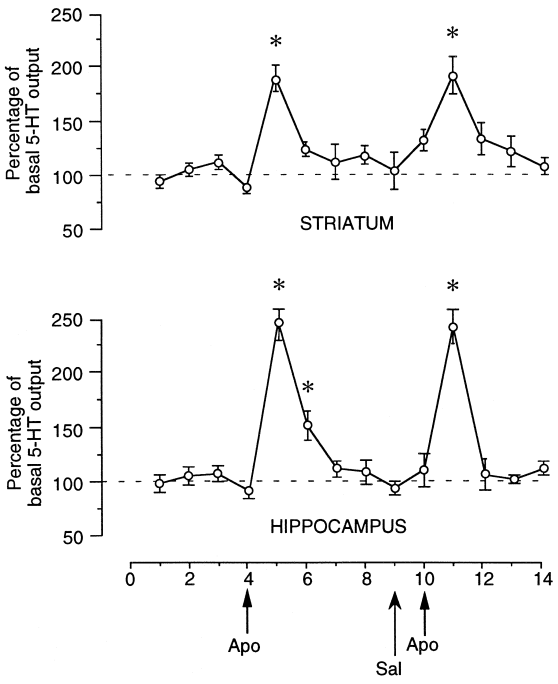

Fig. 1. Time course of the effects of apomorphine (0.5 mg/kg, s.c.) on 5-HT efflux in corpus striatum and hippocampus (20-min samples). The first and second apomorphine injections produced similar increases in 5-HT efflux. Each point represents mean values ± S.E.M. of five to seven animals. Data are expressed as percentages of the four baseline samples. Arrows represent times of apomorphine (Apo) or saline (Sal) injections. * $P < 0.05$ compared to baseline; Student–Newman–Keuls' multiple comparisons test.

As shown in Fig. 2, neither saline nor WAY-100635 (0.1 mg/kg, s.c.) pretreatments altered the 5-HT response to the second apomorphine challenge in either site. In contrast, raclopride, administered either systemically (0.5 mg/kg, s.c.) or via the striatal probe (10 μM) significantly reduced the apomorphine-induced increases in the 5-HT efflux in corpus striatum and hippocampus ($F(39,221) = 3.0$, $P < 0.01$, $n = 21$; $F(39,221) = 3.0$, P

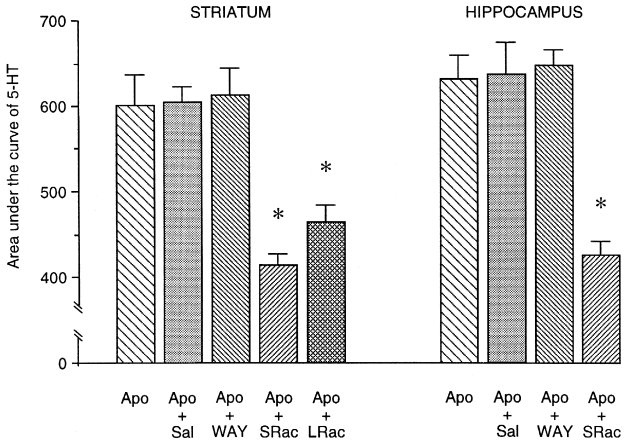

Fig. 2. Effects of pretreatment with a dopamine D_2 or a serotonin 5-HT_{1A} receptor antagonist on apomorphine (Apo, 0.5 mg/kg, s.c.)-induced increases in 5-HT output in the corpus striatum and hippocampus. Abbreviations: saline (Sal, 1 ml/kg, s.c.), WAY-100635 (WAY, 0.1 mg/kg, s.c.), systemic raclopride (SRac, 0.5 mg/kg, s.c.), local raclopride (LRac, 10 μM added to the striatal perfusate). Each column represents mean values ± S.E.M. of five to seven animals. Data are expressed as area under the curve of 5-HT for four consecutive samples. * $P < 0.05$ compared to the control condition (apomorphine alone); Student–Newman–Keuls' multiple comparisons test.

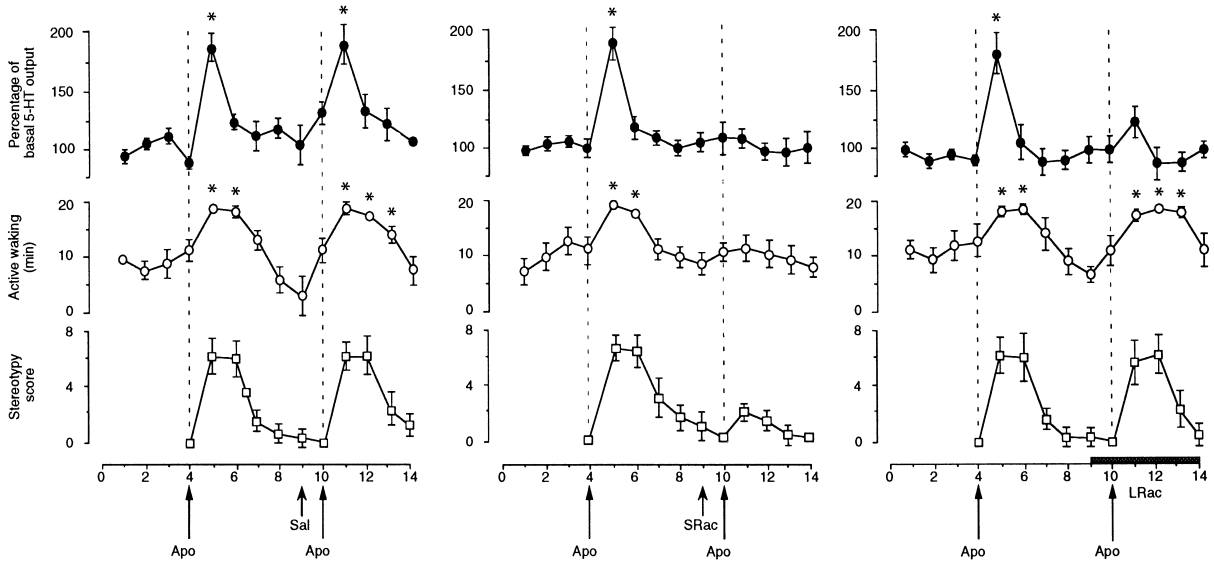

Fig. 3. Summary of the effects of apomorphine on 5-HT efflux in the corpus striatum, behavioral activity and stereotypy. Left panel: effects of apomorphine (0.5 mg/kg, s.c.) after saline pretreatment. Center panel: effects of apomorphine after systemic raclopride pretreatment (0.5 mg/kg, s.c.). Right panel: effects of apomorphine after local dopamine D_2 receptor blockade in corpus striatum with 10 μM raclopride. All abbreviations are as in Fig. 2. Arrows represent times of Apo, SRac or Sal injections, and the horizontal bar represents the time of LRac perfusion through the dialysis probe. Each point represents mean values ± S.E.M. of five to seven animals. Data are expressed as percentages of four baseline samples. $* P < 0.05$ compared to baseline; Student–Newman–Keuls' multiple comparisons test.

< 0.01, $n = 21$, and $F(26,208) = 5.8$, $P < 0.01$, $n = 19$, respectively). Raclopride pretreatment also blocked apomorphine-induced decreases in dopamine in the corpus striatum ($F(39,156) = 2.8$, $P < 0.01$, $n = 16$) and in DOPAC in both areas ($F(13,208) = 5.3$, $P < 0.01$, $n = 20$ and $F(26,195) = 5.7$, $P < 0.01$, $n = 18$, respectively; see Table 1). In the control groups, neither systemic nor local administration of raclopride by itself had an effect on the 5-HT output in either corpus striatum ($F(10,50) = 1.0$, $P = 0.64$, $n = 6$ for local application and $F(10,50) = 0.9$,

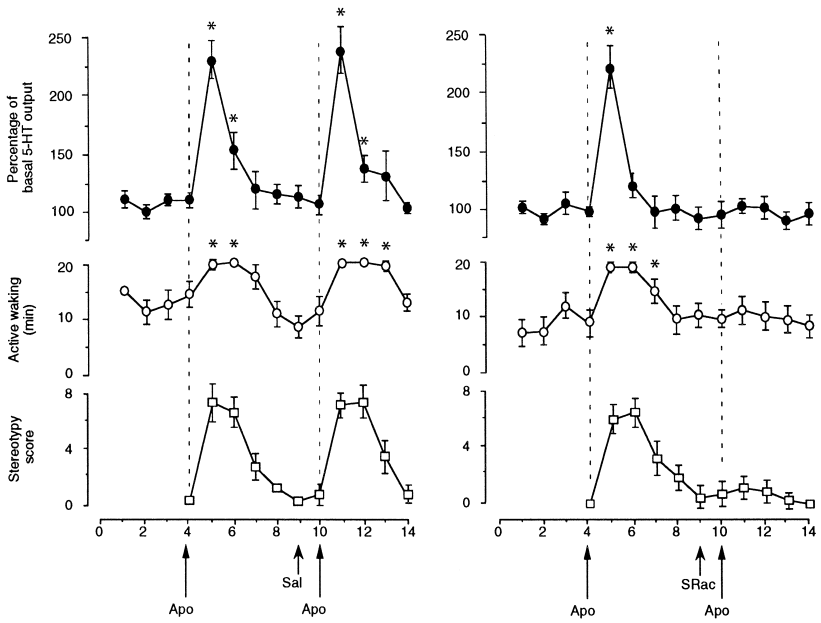

Fig. 4. Summary of the effects of apomorphine on 5-HT efflux in hippocampus, on behavioral activation and stereotypy. Left panel: effects of apomorphine (0.5 mg/kg, s.c.) after saline pretreatment. Right panel: effects of apomorphine after systemic raclopride pretreatment (0.5 mg/kg, s.c.). All abbreviations are as in Fig. 2. Arrows represent times of Apo, SRac or Sal injections. Each point represents mean values ± S.E.M. of five to seven animals. Data are expressed as percentages of the four baseline samples. $* P < 0.05$ compared to baseline; Student–Newman–Keuls' multiple comparisons test.

$P = 0.55$, $n = 6$ for systemic administration) or hippocampus ($F(10,50) = 0.18$, $P = 0.99$, $n = 6$; see Table 1).

Apomorphine challenge led to significant increases in the amount of time animals spent in active waking ($F(12,48) = 4.0$, $P < 0.01$, $n = 5$, and $F(13,39) = 2.9$, $P < 0.01$, $n = 6$ in the corpus striatum group and hippocampus group, respectively). This effect persisted longer than the concomitant increases in the 5-HT output (see Figs. 3 and 4). Systemic raclopride pretreatment completely blocked the behavioral activation produced by apomorphine ($F(12,48) = 14.2$, $P < 0.01$, $n = 5$, and $F(13,39) = 19.8$, $P < 0.01$, $n = 6$ in the corpus striatum group and hippocampus group, respectively). Similar results were obtained for apomorphine-evoked stereotyped behaviors. Administration of 0.5 mg/kg apomorphine resulted in pronounced behavioral stereotypies. Stereotypy ratings in individual animals for the two post-apomorphine samples ranged from 5 to 9, with a mean of 6.2 ± 0.8 ($n = 18$). The stereotyped behavior also lasted longer than the increases in 5-HT output, and was completely blocked by systemic raclopride pretreatment ($F(4,45) = 40.5$, $P < 0.01$, $n = 5$ and $F(6,63) = 48.2$, $P < 0.01$, $n = 7$, in the corpus striatum group and hippocampus group, respectively).

4. Discussion

Systemic administration of apomorphine produced an increase in 5-HT efflux in both hippocampus and corpus striatum, supporting the hypothesis of a facilitatory, rather than an inhibitory, dopaminergic modulation of 5-HT neurotransmission. Several microdialysis studies have also shown that dopamine receptor stimulation, with receptor agonists and/or endogenous dopamine, increased 5-HT efflux in forebrain areas such as frontal cortex (Petty et al., 1994), nucleus accumbens (Li et al., 1996a), corpus striatum (Martín-Ruiz et al., 1997), and hippocampus (Matsumoto et al., 1996). The present results are also in agreement with the electrophysiological studies that reported a stimulatory effect of systemic apomorphine treatment on the firing rate of serotonergic neurons in the dorsal raphe nucleus of behaving cats (Fornal et al., 1996) and anesthetized rats (Martín-Ruiz et al., 1997). The stimulatory effect of dopamine on serotonergic transmission is supported by the ex vivo studies that reported increased 5-HT utilization and/or tissue content produced by dopamine receptor stimulation (Lee and Geyer, 1984; Lee, 1987). Taken together, the biochemical and electrophysiological data provide evidence for the excitatory nature of dopaminergic effect on the serotonergic system.

Dopaminergic facilitation of 5-HT transmission might take place at the level of serotonergic cell bodies, resulting in generalized changes in neurotransmission, and/or in the projection areas, leading to fine regulation of release triggered by local factors in the vicinity of specific nerve terminals. In the present study, local blockade of the striatal dopamine D_2 receptors prevented apomorphine-induced increases in 5-HT output, suggesting that the facilitation of 5-HT transmission takes place primarily at the nerve terminal level. Likewise, application of dopamine receptor agonists via a dialysis probe facilitated 5-HT efflux in frontal cortex (Petty et al., 1994) and hippocampus (Matsumoto et al., 1996), though it failed to produce a change in striatal 5-HT output (Ferré et al., 1994). The increase in 5-HT efflux produced by dopamine receptor agonists was also mimicked by local dopamine uptake blockade (Matsumoto et al., 1996; Li et al., 1996a), suggesting that endogenous dopamine has a stimulatory effect on serotonergic transmission at the nerve terminal level. In the present study, blockade of the 5-HT_{1A} receptors did not alter the effect of apomorphine on either striatal or hippocampal 5-HT efflux, suggesting a lack of involvement of the autoreceptor-mediated negative feedback mechanism in the stimulation of 5-HT efflux produced by apomorphine. Though administration of dopamine receptor agonists elevates firing of the dorsal raphe 5-HT neurons (Fornal et al., 1996; Martín-Ruiz et al., 1997), this does not necessarily entail enhanced transmitter release in the projection areas: for example, WAY-100635, a selective and potent antagonist of 5-HT_{1A} receptors, has no detectable effect on the 5-HT efflux at nerve terminals (Assie and Koek, 1996) despite its pronounced excitatory effects on the firing rate of serotonergic neurons (Fornal et al., 1996). Yet, apomorphine-induced increases in the 5-HT cell firing rate (Fornal et al., 1996), and dorsal raphe 5-HT efflux (Martín-Ruiz et al., 1997) were potentiated by WAY-100635. Taken together, these data indicate that dopaminergic stimulation might have a dual effect: at the somatodendritic level, it leads to increases in both cell firing rate and 5-HT output, while at the level of nerve terminals it produces facilitation of 5-HT efflux that is mostly independent of the increases in firing rate and can be abolished by local receptor blockade.

Blockade of apomorphine-induced responses by raclopride, a compound 1000-fold more selective at dopamine D_2 than at dopamine D_1 receptors (Jackson and Westlind-Danielsson, 1994), suggests that the effects of systemic apomorphine treatment are mediated by dopamine D_2 receptors. It has been previously reported that dopamine D_1 and D_2 receptors can act synergistically (Clark and White, 1987); however, it is unlikely that this mechanism is involved in mediating the apomorphine effect on 5-HT output, since dopamine D_2 receptor antagonists, but not dopamine D_1 receptor antagonists, blocked the apomorphine-induced increases in 5-HT efflux in hippocampus (Matsumoto et al., 1996), as well as in the dorsal raphe nucleus (Ferré and Artigas, 1993). A dopamine D_2 receptor-mediated mechanism might also account for the effects of dopaminergic stimulation at the somatodendritic level, since dopamine D_2 receptor agonist quinpirole increased 5-HT output in the dorsal raphe nucleus, while raclopride

blocked the effect of apomorphine on somatodendritic 5-HT efflux and reduced the firing rate of 5-HT cells (Ferré et al., 1994; Martín-Ruiz et al., 1997). A dopamine D_2 receptor-mediated mechanism, however, does not seem to influence 5-HT efflux under basal physiological conditions, since neither systemic nor local raclopride administration, by itself, affected 5-HT output in the present study.

The effects of apomorphine on dialysate 5-HT content were more pronounced in hippocampus than in the corpus striatum. This difference may be related to the different sources of the serotonergic projections (Jacobs and Azmitia, 1992), as well as to the differential pattern of dopaminergic innervation and receptor density in the corpus striatum as compared to hippocampus (Bouthenet et al., 1987; Jackson and Westlind-Danielsson, 1994). The corpus striatum receives serotonergic innervation from the dorsal raphe nucleus, while hippocampus receives it from both dorsal and median raphe nuclei. These nuclei respond differently to local dopamine D_2 receptor stimulation: 5-HT outflow was increased by local stimulation of dopaminergic receptors in the dorsal (Ferré et al., 1994), but not in the median raphe nucleus (Adell and Artigas, 1997).

In the present study, 5-HT efflux was not affected by a low dose of apomorphine (0.1 mg/kg) that preferentially stimulated presynaptic dopaminergic receptors and failed to evoke any behavioral changes; this is in agreement with the results showing that a low dose of apomorphine did not affect the firing rate of the dorsal raphe 5-HT cells (Fornal et al., 1996). Taken together, these data indicate that the facilitatory effect of apomorphine on 5-HT transmission does not result from the reduced firing rate of dopaminergic neurons produced by dopamine autoreceptor-mediated negative feedback mechanism.

The effects of 0.5 mg/kg of apomorphine, a dose sufficient to produce postsynaptic dopaminergic activation, were characterized by increased locomotor activity and behavioral stereotypies. The intensity of the stereotyped behavior reported here is in good agreement with that previously reported for the same dose of the drug (Costall et al., 1972). The stereotypy score may serve as an independent measure of the postsynaptic action of apomorphine and as an indication of its time course, since the stereotypies produced by dopamine receptor agonists are largely dependent upon the dopamine receptor stimulation in the caudate nucleus (Jackson and Westlind-Danielsson, 1994). Analysis of stereotypies and the increases in general behavioral activation produced by apomorphine demonstrates that both responses had a more prolonged time course than the concurrent increases in 5-HT output. There exists a tight coupling between the level of behavioral arousal (as indicated by the amount of time spent in active waking) and the activity of the serotonergic system in terms of both cell firing (Jacobs and Fornal, 1997) and extracellular 5-HT output (Kalen et al., 1989; Mendlin et al., 1996; Rueter and Jacobs, 1996). The dissociation in the time course of the effects of dopaminergic stimulation on behavioral activation and transmitter efflux indicates that the biochemical and behavioral responses are parallel and independent consequences of the apomorphine treatment; in other words, increases in the 5-HT output are a direct outcome of dopaminergic stimulation rather than a by-product of the general behavioral activation produced by the drug. Furthermore, the magnitude of the increases in 5-HT efflux produced by changes in behavioral state alone (Kalen et al., 1989; Mendlin et al., 1996; Rueter and Jacobs, 1996) is lower than that observed in the present study (30–60% vs. 75–120%). In addition, the studies where apomorphine was administered via the dialysis probe demonstrate a facilitation of the 5-HT efflux in frontal cortex (Petty et al., 1994) and hippocampus (Matsumoto et al., 1996) in the absence of behavioral changes. This interpretation is strengthened by the fact that local blockade of the dopamine D_2 receptors at the nerve terminal level in the present study did not affect the increases in behavioral activation or stereotypies, but significantly reduced apomorphine-induced increase in 5-HT efflux; the residual small increase in 5-HT output can probably be accounted for by the changes in behavioral state.

In summary, the present study demonstrated that systemic administration of apomorphine facilitated 5-HT output in both the corpus striatum and hippocampus. This effect was mediated by dopamine D_2 receptors, but not by 5-HT_{1A} receptors, and it was abolished by local blockade of dopamine D_2 receptors in the corpus striatum. Apomorphine-produced behavioral activation did not seem to account for the neurochemical effects of the drug, suggesting that these effects are attributable to the activation of dopamine receptors. Based on these pharmacological results, our laboratory is currently examining the influence of dopamine on 5-HT output under physiological conditions.

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