ELSEVIER

Pharmacology, Biochemistry and Behavior 71 (2002) 627–633

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Central 5-HT_4 receptors and dopamine-dependent motor behaviors: Searching for a functional role

Philippe De Deurwaerdère[a], Luigi Cervo[b], Luis Stinus[a], Umberto Spampinato[a,*]

[a]*Laboratoire Neuropsychobiologie des Désadaptations, UMR-CNRS 5541, Université Victor Segalen Bordeaux 2, B.P. 31, 146 rue Léo-Saignat, 33077 Bordeaux Cedex, France*

[b]*Istituto di Ricerche Farmacologiche "Mario Negri," via Eritrea, 62, 20157 Milan, Italy*

Received 31 May 2001; received in revised form 16 August 2001; accepted 1 September 2001

Abstract

In this study, we evaluated the role of central 5-HT_4 receptors in the control of motor behaviors related to change of nigrostriatal dopamine (DA) transmission, namely, stereotyped behavior and catalepsy in rats. Indeed, given that 5-HT_4 receptors indirectly modulate nigrostriatal DA neuron activity, we hypothesized that these receptors would regulate nigrostriatal DA transmission in the basal ganglia, and consequently, associated motor responses. Stereotypy was induced either by an acute administration of apomorphine (0.3 and 1.5 mg/kg sc), or by a single morphine administration (15 mg/kg sc) in chronically morphine-treated (15 mg/kg sc, twice daily for 10 days) rats. Catalepsy was induced by the typical neuroleptic haloperidol (HAL; 1 mg/kg sc). The selective 5-HT_4 antagonist, GR 125487 (1 mg/kg ip), modified neither apomorphine- nor morphine-induced stereotypy. HAL-induced catalepsy, while reduced by the systemic administration of the 5-HT_{1A} agonist 8-OH-DPAT (0.1 mg/kg sc), was insensitive to GR 125487, systemically (1, 3, 10 mg/kg ip) or locally (20 and 40 nmol/20 μl) administered into the third ventricle. Also, HAL-induced catalepsy was not affected by the selective 5-HT_4 antagonist GR 113808 (3 mg/kg ip). The obtained results indicate that 5-HT_4 receptor antagonism does not modulate motor behaviors related to change of striatal DA transmission.

Keywords: Haloperidol; Catalepsy; Stereotypy; Apomorphine; Morphine; 5-HT_4 receptors; GR 125487; GR 113808

1. Introduction

It is now well established that the central serotonergic (5-HT) system modulates nigrostriatal dopamine (DA) neuron activity, and the knowledge of this interaction has been pointed out as a promising avenue for improved therapy of various neuropsychiatric diseases such as schizophrenia or Parkinson's disease (Barnes and Sharp, 1999; Kapur and Remington, 1996). In this context, 5-HT_4 receptors, which have been described within the central nervous system (CNS) of several mammalian species (Dumuis et al., 1988; Grossman et al., 1993), have been proposed as an important target in mediating the modulatory effects of 5-HT on DA neuron activity. Indeed, anatomical studies have shown that these receptors are highly enriched in DA-innervated regions of the basal ganglia (Jakeman et al., 1994), and converging electrophysiological and biochemical evidences have shown that 5-HT_4 receptors exert an excitatory control on nigrostriatal DA function (Bonhomme et al., 1995; De Deurwaerdère et al., 1997; Lucas et al., 2001; Pozzi et al., 1995; Steward et al., 1996; Thorré et al., 1998).

Given that 5-HT_4 receptors are not expressed by nigrostriatal DA neurons (Compan et al., 1996; Patel et al., 1995), the modulation they exert on DA neuron activity is indirect (De Deurwaerdère et al., 1997; Steward et al., 1996). It is therefore conceivable that 5-HT_4 receptors may regulate the level of nigrostriatal DA transmission in the basal ganglia and, consequently, motor behaviors related to change of DA transmission, such as catalepsy and stereotypy (Dunnett and Robbins, 1992). The functional significance of this interaction has been recently highlighted by a clinical report indicating that the nonselective 5-HT_4 agonist and gastrointestinal prokinetic agent cisapride worsen parkinsonism (Sempere et al., 1995), but only a few studies (Pires et al.,

* Corresponding author. Tel.: +33-557-57-1544; fax: +33-556-90-0278.

E-mail address: umberto.spampinato@lnpb.u-bordeaux2.fr (U. Spampinato).

0091-3057/02/$ – see front matter
PII: S0091-3057(01)00703-1

1993; Reavill et al., 1998; Ward and Dorsa, 1999) have investigated the functional role of 5-HT_4 receptors in nigrostriatal DA-mediated behaviors.

Thus, in the present study, we have examined, by using the potent and selective 5-HT_4 receptor antagonists GR 125487 or GR 113808 (Consolo et al., 1994; Letty et al., 1997), the role of 5-HT_4 receptors in DA-dependent motor behaviors related to either blockade (catalepsy) or enhancement (stereotypy) of nigrostratal DA transmission in rats (Dunnett and Robbins, 1992). Catalepsy was induced by the systemic administration of the typical neuroleptic haloperidol (HAL). Stereotyped behavior was induced either by an acute administration of the DA agonist apomorphine, or by a single morphine administration in chronically morphine-treated rats. These experimental conditions lead to a direct and indirect stimulation of postsynaptic DA receptors, respectively (Cervo et al., 1981; Themann et al., 1984), and are known to be sensitive to 5-HT modulation (Bendotti et al., 1980; Cervo et al., 1981).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 300–340 g (IFFA CREDO, France), or 150–175 g (Charles River, Italy) were used in these studies. Animals were housed at constant room temperature (21 ± 2 °C) and relative humidity (60%) with a regular 12-h light/dark cycle (dark from 8:00 p.m.) and had free access to food and water. All animals were allowed to adapt to laboratory conditions for at least 1 week and were handled for 5 min per day during this adaptation period. All experiments were conducted in conformity with the institutional guidelines that are in compliance with the national (in France: no. 87-848; in Italia: D.L. n. 116, G.U., suppl. 40, 18 Febbraio 1992, Circolare No. 8, G.U., 14 Luglio 1994) and international laws and policies (EEC Council Directive No 86/609, OJL 358, 1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

2.2. Catalepsy experiments

2.2.1. Surgery

To allow the intracerebroventricular administration of the 5-HT_4 receptor antagonist GR 125487 in some experiments, a cannula was implanted according to the experimental procedure of Oberling et al. (1993) with some modification. Briefly, rats originally weighing 320 g were anaesthetized with chloral hydrate (600 mg/kg ip) and placed in a stereotaxic frame (David Kopft instruments) with the incisor bar set at +5 mm above the interaural line (Pellegrino and Cushman, 1967). A stainless steel cannula (o.d. = 0.6 mm; i.d. = 0.32 mm) was implanted in the third ventricle (AP: −1.8; L = 0; relative to bregma; V: −4.2 relative to the dura matter). The cannula was secured to the skull with dental cement and anchored to stainless steel mounting screws placed in the skull. A removable stainless steel stylet (o.d. = 0.29 mm) was placed inside the cannula to prevent its occlusion. Following this surgery, rats, housed individually, were allowed 10 days of recovery before behavioral testing.

2.2.2. Pharmacological treatments

HAL, diluted in sterile saline (NaCl 0.9%), was subcutaneously injected at the dose of 1 mg/kg. In the case of systemic administration of 5-HT agents, 8-OH-DPAT (0.1 mg/kg) or GR 125487 (1, 3 or 10 mg/kg ip) dissolved in sterile NaCl 0.9%, were administered 150 min after HAL. In another set of experiments, GR 113808 (3 mg/kg ip), also dissolved in sterile NaCl 0.9%, was administered 170 min after HAL. 8-OH-DPAT and 5-HT_4 antagonists administration times and routes were chosen on the basis of the pharmacokinetic properties of these drugs, such that compounds were at their pharmacodynamic maximum when the cataleptic effect of HAL was maximal (Neal-Beliveau et al., 1993; Letty et al., 1997; Lucas et al., 1997, 2001; Silvestre et al., 1996).

In the case of intracerebroventricular administration, awake rats were gently hand-held to remove the stylet and to insert a stainless steel injection cannula (o.d. = 0.30 mm; i.d. = 0.18 mm) that extended 1.5 mm beyond the tip of the guide cannula. The injection cannula was connected to a 50-μl microsyringe (Hamilton) via a polyethylene tubing and animals were placed in a circular plastic chamber. Freely moving animals were delivered a total volume of 20 μl over 2 min of GR 125487 (20 or 40 nmol) or its vehicle (sterile saline) 175 min after the administration of HAL or its vehicle. The connection was kept in place for an additional 60 s to allow diffusion of the drug. At the end of the experiment, the animals were sacrificed by lethal anesthesia (chloral hydrate) and microinjected with dilute blue ink (2 μl) to verify the correct location of the injection.

2.2.3. Behavioral testing

Experiments were carried out in a sound-attenuated room between 10:00 a.m. and 5:00 p.m. The animals were brought from the vivarium to the testing room 1 h before the experiment to allow habituation to the new environment. Catalepsy was measured 60, 120, 150, 180, 210, and 240 min after HAL administration by gently placing both forepaws of the rat on the top of a wooden block (height = 9 cm) with the hindlimbs abducted (Lucas et al., 1997). In the case of intracerebroventricular administration, catalepsy induced by HAL was evaluated each 15 min after 5-HT_4 antagonist injection (180, 195, 210, 225, and 240 min after HAL administration). The intensity of catalepsy was assessed by counting the time the animal remained in this position with a maximal "cut-off" of 300 s. Catalepsy

measurement was performed by an observer blind to the drug schedule administration.

2.3. Stereotypy experiments: pharmacological treatments and behavioral testing

Experiments were carried out in a sound-attenuated, uniformly lit room between 9.00 a.m. and 1:00 p.m. At the beginning of the experiments, before any drug treatment, the animals were placed in clear Plexiglas observation cages with steel grid floors for 1 h to allow habituation to the new environment.

Stereotypies induced by apomorphine and morphine were studied in separate experiments. In these experiments, GR 125487 was administered intraperitoneally at the dose of 1 mg/kg on the basis of recent biochemical findings showing its effectiveness in modulating central DA neuron activity (Lucas et al., 2001). In a first experiment, separate groups of eight rats each received 1 mg/kg GR 125487 or its vehicle 15 min before a subcutaneous injection of 0.3 or 1.5 mg/kg apomorphine. Apomorphine-induced stereotyped behavior was scored from 0 to 4 according to Costall and Naylor (1973): 0 (*no stereotyped behavior*); 1 (*periodic sniffing*); 2 (*continuous sniffing*); 3 (*periodic licking or biting of the grid*); 4 (*continuous licking and biting of the grid*). Stereotypy score was evaluated during 15 s observation every 15 min.

In a second experiment, according to the procedure previously described by Cervo et al. (1981), 32 animals received two daily injections (at 10:00 a.m. and 5:00 p.m.) of 15 mg/kg morphine subcutaneously for 10 days. Twenty-four hours after the last morphine injection, animals were injected intraperitoneally with 1 mg/kg GR 125487 or its vehicle 15 min before a challenge dose of 15 mg/kg morphine or vehicle. Chronic morphine-induced stereotypy was evaluated according to Cervo et al. (1981): animals showing intense licking and/or biting during a 30-s period of observation 1 h after the narcotic were considered positive. In all cases, measurements were performed by an observer unaware of the treatment.

2.4. Statistical analysis

The intensity of catalepsy, expressed in seconds, was determined as the mean ± S.E.M. of the time of immobility. Results were analyzed for each time point by the Kruskall–Wallis nonparametric ANOVA or, when appropriate, by the Mann–Whitney U test. Stereotyped behavior elicited by apomorphine was expressed as the median value with interquartile ranges of the stereotypy score for each time point. Data were analyzed by the Mann–Whitney U test for each time point. Finally, morphine-induced stereotyped behavior was expressed as the proportion of animals exhibiting the behavior, and analyzed by the Fisher's Exact Test. In all cases, $P < .05$ was chosen as a criterion for statistical significance.

2.5. Drugs

The following drugs were used: GR 113808 ([1-[2(methylsulfonylamino)ethyl]4-piperidinyl]methyl-1methyl-1-*H*-indole-3-carboxylate) hydrochloride, GR 125487 {[1-[2-(methylsulfonylamino)ethyl]-4-piperidinyl]methyl-5-fluoro-2-methoxy-1-*H*-indole-3-carboxylate} sulphamate, kindly donated by Dr. Bain (Glaxo Research Group, Ware, Hertfordshire, UK); (±)8-OH-DPAT hydrobromide, apomorphine hydrochloride, morphine hydrochloride (Research Biochemicals International, Natick, MA, USA), and HAL as the commercially available solution (Haldol 5 mg/ml, Janssen Pharmaceutica, Beerse, Belgium). All other chemicals and reagents were the purest commercially available (Merck, Prolabo).

In all cases, drug solutions used in the study were freshly prepared, subcutaneous or intraperitoneal injections were performed in a volume of 1 or 2 ml/kg body weight, respectively. HAL and 8-OH-DPAT doses refer to their free-base weight.

3. Results

3.1. Effect of GR 125487 on apomorphine- and morphine-induced stereotyped behavior

As previously reported (Fray et al., 1980), 0.3 and 1.5 mg/kg apomorphine induced a dose-dependent increase in stereotyped behavior, reaching its maximum 15 and 30 min after its administration, respectively (Fig. 1). GR 125487 (1 mg/kg ip) did not alter the effect of either 0.3 or 1.5 mg/kg apomorphine (Mann–Whitney U test, $P > .05$ for each time point).

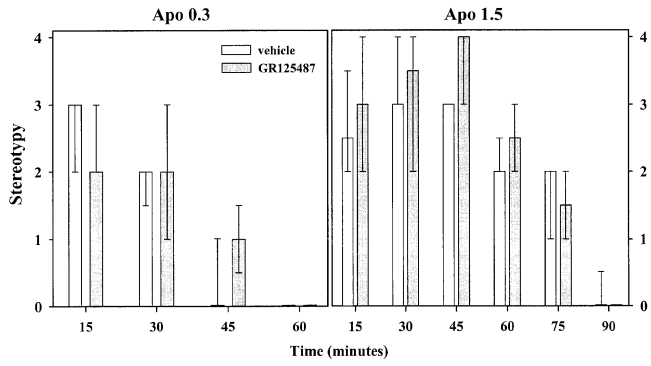

Fig. 1. Effect of GR 125487 on apomorphine-induced stereotyped behavior. Histograms represent the median value of the stereotypy score of eight rats per treatment group in each experiment. Bars refer to the interquartile range of this distribution. Apomorphine (Apo) was administered subcutaneously (0.3 and 1.5 mg/kg; Time 0), 15 min after an intraperitoneal injection of either 1 mg/kg GR 125487 or vehicle. Stereotyped behavior was evaluated during a 15-s period observation every 15 min according to Costall and Naylor (1973). Data were analyzed by the Mann–Whitney U test.

Table 1
Effect of GR 125487 on morphine-induced stereotyped behavior

	Proportion of animals showing stereotypy	
Treatment	Saline	Morphine
Saline	0/6	7/8
GR 125487	0/6	7/8

Data are the proportion of animals exhibiting continuously licking or biting out of the total number of animals tested. Stereotypy score was evaluated during a 15-s observation 1 h after morphine injection according to Cervo et al. (1981). Morphine (15 mg/kg sc) was given twice daily for 10 days. Twenty-four hours after the last morphine injection, rats ($n=6-8$ per group) received either GR 125487 (1 mg/kg ip) or vehicle (saline) 15 min before a challenge dose of morphine (15 mg/kg sc) or vehicle (saline). Data were analyzed by the Fischer's Exact Test.

Morphine-induced stereotyped behavior was observed in seven of the eight rats chronically treated with morphine (Table 1). GR 125487, without effect by itself, did not modify the ability of morphine to induce stereotypy ($P>.05$, Fisher's Exact Test).

3.2. Effect of 8-OH-DPAT and 5-HT$_4$ antagonists on HAL-induced catalepsy

As shown in Fig. 2, 1 mg/kg HAL induced a strong cataleptic state starting at the first hour, and reaching a maximal plateau 3 h (240 ± 25 s) after its administration. Control animals (vehicle + vehicle) did not display catalepsy, as they remained less than 5 s on the block at each time point ($n=5$, data not shown).

As illustrated in Fig. 2A, the 5-HT$_{1A}$ agonist 8-OH-DPAT, administered 2.5 h after HAL, antagonized catalepsy at 3 h ($P<.001$; Mann–Whitney U test), 3.5 h ($P<.05$), but not at 4 h after neuroleptic injection. As reported previously (Lucas et al., 1997), 8-OH-DPAT (0.1 mg/kg sc) elicited by itself some components of the "serotonergic syndrome," such as flat-body posture and forepaw treading occurring within 30–45 min after 8-OH-DPAT administration (Tricklebank et al., 1984).

The administration of the 5-HT$_4$ antagonist GR 125487, whatever the dose administered (1, 3, or 10 mg/kg ip), did not affect catalepsy induced by HAL (Kruskall–Wallis ANOVA, $P>.05$; $n=10-12$ animals per dose; Fig. 2B). Also, as depicted in Fig. 2C, intracerebroventricular administration of GR 125487 at 20 and 40 nmol failed to affect HAL-induced catalepsy (Kruskall–Wallis ANOVA, $P>.05$; $n=4-6$ animals per dose). Similar results were also observed in the presence of the 5-HT$_4$ antagonist GR 113808. Thus, HAL-induced catalepsy was not affected by 3 mg/kg GR 113808, administered intraperitoneally 170 min after HAL injection [Time 180: 283 ± 11 and 251 ± 39 s; Time 210: 291 ± 9 and 25432 s; Time 240: 297 ± 3 and 298 ± 2 s in HAL + saline ($n=10$) and HAL + GR 113808 ($n=8$) groups, respectively; $P>.05$, Mann–Whitney U test; data not shown]. Finally, in all cases, animals treated with 5-HT$_4$ antagonists alone (vehicle + 5-HT$_4$ antagonist groups) did not display catalepsy, as they remained less than 5 s on the block at each time point (data not shown).

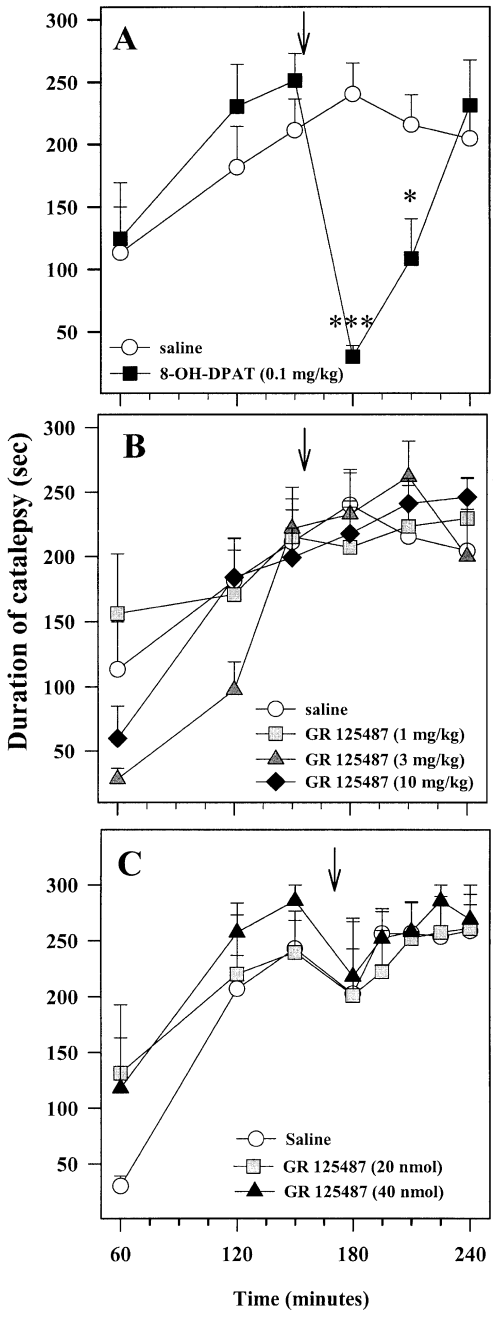

Fig. 2. Effect of 8-OH-DPAT and GR 125487 on the duration of HAL-induced catalepsy measured at different time points after HAL administration. In each experiment, results are the mean ± S.E.M of the time spent by animals on the wooden block. 8-OH-DPAT (0.1 mg/kg sc) (A) and GR 125487 (1, 3, and 10 mg/kg ip) (B) were administered 2.5 h after the subcutaneous injection of 1 mg/kg HAL ($n=10-12$ animals per treatment group in each experiment). GR 125487 was also administered intracerebroventricularly at 20 and 40 nmol 2.5 h after HAL (C) ($n=4-6$ animals per treatment group). Data were analyzed by the Mann–Whitney U test (A) or by the Kruskall–Wallis ANOVA (B and C). * $P<.05$; *** $P<.001$ vs. HAL alone, Mann–Whitney U test.

4. Discussion

The present study was aimed to address the role of central 5-HT_4 receptors in motor behaviors related to change of nigrostriatal DA transmission. This hypothesis took place on the basis of converging anatomical, electrophysiological, and biochemical evidence showing that 5-HT_4 receptors are highly concentrated in DA containing brain areas, and modulate nigrostriatal DA neuron activity. The obtained results, showing that 5-HT_4 antagonism affects neither apomorphine- and morphine-induced stereotypy nor HAL-induced catalepsy in rats, indicate that 5-HT_4 receptor function is not associated with DA-dependent motor behaviors.

Stereotyped behavior is thought to be consequent to an increase in nigrostriatal DA transmission (Dunnett and Robbins, 1992) and previous data in the literature have suggested that the influence of 5-HT_4 receptors on central DA neuron function is noticeable in the case of increased DA transmission (Lucas et al., 2001; Pozzi et al., 1995). In the present study, stereotyped behavior was achieved by using two different pharmacological treatments, apomorphine and morphine, leading to an enhancement of striatal DA transmission via distinct mechanisms. Thus, whereas apomorphine elicits stereotypy by directly stimulating postsynaptic DA receptors (Costall and Naylor, 1973), morphine-induced stereotyped behavior is thought to be consequent to increased striatal DA release (Cervo et al., 1981; Spampinato et al., 1983). We found that GR 125487 did not affect stereotyped behavior induced by either 0.3 or 1.5 mg/kg apomorphine. This result suggests that 5-HT_4 receptors are not involved in stereotyped behavior whatever the degree of increase in nigrostriatal DA transmission. Furthermore, it appears unlikely that, although DA and 5-HT_4 receptors share common targets within the striatum, namely striatal GABAergic neurons (Compan et al., 1996; Ward and Dorsa, 1999), 5-HT_4 receptors may modify the efficiency of striatal DA transmission by acting at the level of intracellular transduction systems. Previous studies have suggested that stereotyped behavior elicited by direct stimulation of postsynaptic DA receptors is less sensitive to 5-HT regulation compared to that induced by increased DA neuron activity (Jori et al., 1974), such as morphine-induced stereotypy (Cervo et al., 1981). However, our finding that GR 125487 has no influence on morphine-induced stereotypy indicates that DA-mediated behaviors associated with increased DA release are also insensitive to 5-HT_4 antagonism. This result is quite surprising when considering that 5-HT_4 antagonism attenuates morphine-induced change of nigrostriatal DA neuron activity (Pozzi et al., 1995). However, it is worth noting that an action on striatal DA release may not be critical to modify stereotyped behavior, in that change of striatal DA release is not strictly linked to change of motor responses. For instance, the preferential 5-HT_{2A} antagonist amperozide, while reducing amphetamine-induced striatal DA release, did not affect amphetamine-induced stereotypy (Ichikawa and Meltzer, 1992). Nevertheless, it cannot be excluded that adaptive changes consequent to chronic morphine administration may prevent the ability of 5-HT_4 receptors to modulate DA neuron function.

The role of 5-HT_4 receptors has been also studied in the catalepsy induced by HAL, an experimental condition related to a blockade in striatal DA transmission (Dunnett and Robbins, 1992; Sanberg, 1980). Indeed, numerous experimental data indicate that this behavior is sensitive to 5-HT regulation, and converging evidence indicates that a decrease in central 5-HT transmission reduces neuroleptic-induced catalepsy (Soubrié et al., 1984). As previously reported (Hicks, 1990; Neal-Beliveau et al., 1993), we found that 0.1 mg/kg 8-OH-DPAT abolished HAL-induced catalepsy. In contrast, consistent with a recent study (Ward and Dorsa, 1999), GR 125487, over a wide range of doses known to be effective in the CNS (Ge and Barnes, 1996; Letty et al., 1997; Lucas et al., 2001), did not affect HAL-induced catalepsy. A similar result was observed with the potent 5-HT_4 antagonist GR 113808, used at a dose that has been shown to reduce anxiolytic-like activity in the light/dark test (Silvestre et al., 1996). In addition, intracerebroventricular administration of GR 125487, at concentrations known to block central 5-HT_4 receptors (Consolo et al., 1994), also failed to affect catalepsy. This latter result further excludes the possibility that the lack of effect of systemic GR 125487 administration on HAL-induced catalepsy could be related to peripheral pharmacokinetic interaction between these compounds. The anticataleptic effect of 8-OH-DPAT has been related to a massive decrease in 5-HT release in various brain areas consequent to somatodendritic 5-HT_{1A} autoreceptors stimulation (Bonvento et al., 1992; Invernizzi et al., 1988). However, it is worth noting that 5-HT_4 receptor antagonists including GR 125487 also reduce 5-HT release in various brain regions (Ge and Barnes, 1996; Thorré et al., 1998). This common feature shared by 5-HT_{1A} agonist and 5-HT_4 antagonist suggests that factors other than the reduction of 5-HT release may account for the anticataleptic property of 5-HT agents. As debated already in the literature (Lucas et al., 1997), the anticataleptic effect of 8-OH-DPAT might reflect the influence of behaviors such as forepaw treading/flat-body posture elicited by 8-OH-DPAT (Tricklebank et al., 1984; Higgins and Elliott, 1991).

Altogether, our results indicate that 5-HT_4 receptor blockade is not able to modulate behaviors related to changes of nigrostriatal DA transmission. This finding, which likely reflects the low level of endogenous tone on the central 5-HT_4 receptors (Barnes and Sharp, 1999), is in line with a recent report by Reavill et al. (1998) showing that circling behavior induced by amphetamine in unilateral DA-denervated rats is not affected by 5-HT_4 antagonism. These negative behavioral observations are quite surprising when considering the large body of

biochemical and electrophysiological evidence supporting a role for 5-HT_4 receptors in modulating nigrostriatal DA neuron function (De Deurwaerdère et al., 1997; Lucas et al., 2001; Thorré et al., 1998). Nevertheless, biochemical studies also point out that 5-HT_4 receptors require specific conditions to operate, in that their influence on DA neuron function is not observed in all the experimental conditions studied. Thus, at variance with the local administration into the striatum or the substantia nigra (De Deurwaerdère et al., 1997; Steward et al., 1996; Thorré et al., 1998), systemic administration of nonselective 5-HT_4 agonists alter neither DA release nor the expression of the proto-oncogene c-*fos* in the rat striatum (Taylor and Routledge, 1996; Ward and Dorsa, 1999). Also, systemic administration of selective 5-HT_4 antagonists, while reducing morphine- and HAL-induced striatal DA release, does not modify the release of DA elicited by amphetamine or cocaine (Lucas et al., 2001; Pozzi et al., 1995; Spampinato et al., unpublished observations).

The functional significance of the 5-HT_4 receptor-dependent modulation of DA function becomes more puzzling when looking at studies assessing the role of these receptors at the level of mesolimbic DA system, where a quite opposite picture is observed. Thus, whereas electrophysiological and neurochemical studies provide no evidence for a role of 5-HT_4 receptors in the control of mesoaccumbal DA neuron function (Lucas et al., 2001; Pozzi et al., 1995), most, but not all (Reavill et al., 1998), behavioral studies report that 5-HT_4 antagonists modulate behaviors classically related to mesolimbic DA transmission (Bisaga et al., 1993; McMahon and Cunningham, 1999; Panocka et al., 1995). Although these findings do not provide a clear insight into the mechanisms underlying the recruitment of 5-HT_4 receptors in the control of DA neuron function, they emphasize the need to further explore this interaction to determine the specific and permissive factors leading this regulatory process to occur (Lucas et al., 2001).

In summary, our results indicate that 5-HT_4 receptor antagonism has no influence on motor behaviors associated with change of nigrostriatal DA transmission. However, studies with selective and brain penetrant 5-HT_4 agonists are warranted to further assess the functional significance of the 5-HT_4 receptor-dependent modulation of nigrostriatal DA pathway activity, and to ascertain their putative role in the pathophysiology of neuropsychiatric disorders related to central DA neuron dysfunction (Sempere et al., 1995).

Acknowledgments

This work was supported by grants from CNRS and Bordeaux 2 University. The Association France Parkinson is gratefully acknowledged for its financial support. The authors wish to thank Charlotte Blance Ekalle-Soppo for technical assistance and Dr. G. Goodall for linguistic assistance.

References

Barnes NM, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology 1999;38:1083–152.

Bendotti C, Borsini F, Zanini MG, Samanin R, Garattini S. Effect of fenfluramine and norfenfluramine stereoisomers on stimulant effects of D-amphetamine and apomorphine in the rat. Pharmacol Res 1980;12:567–74.

Bisaga A, Sikora J, Kostowski W. The effect of drugs interacting with serotonergic $5HT_3$ and $5HT_4$ receptors on morphine place conditioning. Pol J Pharmacol 1993;45:513–9.

Bonhomme N, De Deurwaerdère P, Le Moal M, Spampinato U. Evidence for 5-HT_4 receptor subtype involvement in the enhancement of striatal dopamine release induced by serotonin: a microdialysis study in the halothane-anesthetized rats. Neuropharmacology 1995;34:269–79.

Bonvento G, Scatton B, Claustre Y, Rouquier L. Effects of local injection of 8-OH-DPAT into the dorsal or median raphé nuclei on extracellular levels of serotonin in serotonergic projection areas in the rat brain. Neurosci Lett 1992;132:101–4.

Cervo L, Romandini S, Samanin R. Evidence of serotonin–dopamine involvement in the inhibition of D-amphetamine stereotypy and appearance of stereotyped movements found respectively after acute and long-term treatment with morphine and methadone in rats. Life Sci 1981;29:2585–91.

Compan V, Daszuta A, Salin P, Sebben M, Bockaert J, Dumuis A. Lesion study of the distribution of serotonin 5-HT_4 receptors in rat basal ganglia and hippocampus. Eur J Neurosci 1996;8:2591–8.

Consolo S, Arnaboldi S, Giorgi S, Russi G, Ladinski H. 5-HT_4 receptor facilitates acetylchoxalic dopaminergic systems in the mediation of apomorphine-stereotyped behaviour. Eur J Pharmacol 1973;24:8–24.

De Deurwaerdère P, L'hirondel M, Bonhomme N, Lucas G, Cheramy A, Spampinato U. Serotonin stimulation of 5-HT_4 receptors indirectly enhances in vivo dopamine release in the rat striatum. J Neurochem 1997;68:195–203.

Dumuis A, Bouhehal R, Sebben M, Cory R, Bockaert J. A nonclassical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system. Mol Pharmacol 1988;34:880–7.

Dunnett SB, Robbins TW. The functional role of mesotelencephalic dopamine systems. Biol Rev 1992;67:491–518.

Fray PJ, Sahakian BJ, Robbins TW, Koob GF, Iversen SD. An observational method for quantifying the behavioural effects of dopamine agonists: contrasting effects of D-amphetamine and apomorphine. Psychopharmacology (Berlin) 1980;69:253–9.

Ge J, Barnes NM. 5-HT_4 receptor-mediated modulation of 5-HT release in the rat hippocampus in vivo. Br J Pharmacol 1996;117:1475–80.

Grossman CJ, Kilpatrick GJ, Bunce KT. Development of a radioligand binding assay for 5-HT_4 receptors in guinea-pig and rat brain. Br J Pharmacol 1993;109:618–24.

Hicks PB. The effect of serotoninergic agents on haloperidol-induced catalepsy. Life Sci 1990;47:1609–15.

Higgins GA, Elliott PJ. Differential behavioural activation following intra-raphe infusion of 5-HT_{1A} receptor agonists. Eur J Pharmacol 1991;193:351–6.

Ichikawa J, Meltzer HY. Amperozide, a novel antipsychotic drug, inhibits the ability of D-amphetamine to increase dopamine release in vivo in rat striatum and nucleus accumbens. J Neurochem 1992;58:2285–91.

Invernizzi RW, Cervo L, Samanin R. 8-Hydroxy-2-(di-*n*-propylamino) tetralin, a selective $serotonin_{1A}$ receptor agonist, blocks haloperidol-induced catalepsy by an action on raphe nuclei medianus and dorsalis. Neuropharmacology 1988;27:515–8.

Jakeman LB, To ZP, Eglen RM, Wong EHF, Bonhaus DW. Quantitative

autoradiography of 5-HT_4 receptors in brains of three species using two structurally distinct radioligands, [^{3}H]GR113808 and [^{3}H]BIMU-1. Neuropharmacology 1994;33:1027–38.

Jori A, Cecchetti G, Ghezzi D, Samanin R. Biochemical and behavioral antagonism between fenfluramine and apomorphine in rats. Eur J Pharmacol 1974;26:179–83.

Kapur S, Remington G. Serotonin–dopamine interaction and its relevance to schizophrenia. Am J Psychiatry 1996;153:466–76.

Letty S, Child R, Dumuis A, Pantaloni A, Bockaert J, Rondouin G. 5-HT_4 receptors improve social olfactory memory in the rat. Neuropharmacology 1997;36:681–7.

Lucas G, Bonhomme N, De Deurwaerdère P, Le Moal M, Spampinato U. 8-OH-DPAT, a 5-HT_{1A} agonist and ritanserin, a 5-$HT_{2A/C}$ antagonist, reverse haloperidol-induced catalepsy in rats independently of striatal dopamine release. Psychopharmacology 1997;131:57–63.

Lucas G, Di Matteo V, De Deurwaerdère P, Porras G, Martin-Ruiz R, Artigas F, Esposito E, Spampinato U. Neurochemical and electrophysiological evidence that 5-HT_4 receptors exert a state-dependent facilitatory control in vivo on nigrostriatal, but not mesoaccumbal dopaminergic function. Eur J Neurosci 2001;13:889–98.

McMahon LR, Cunningham KA. Antagonism of 5-hydroxytryptamine$_4$ receptors attenuates hyperactivity induced by cocaine: putative role for 5-hydroxytryptamine$_4$ receptors in the nucleus accumbens shell. J Pharmacol Exp Ther 1999;291:300–7.

Neal-Beliveau BS, Joyce JN, Lucki I. Serotonergic involvement in haloperidol-induced catalepsy. J Pharmacol Exp Ther 1993;265:207–17.

Oberling P, Stinus L, Le Moal M, Simonnet G. Biphasic effect on nociception and antiopiate activity of the neuropeptide FF (FLFQPQRFamide) in the rat. Peptides 1993;14:919–24.

Panocka I, Ciccocioppo R, Polidori C, Pompei P, Massi M. The 5-HT4 receptor antagonist, GR113808, reduces ethanol intake in alcohol-preferring rats. Pharmacol, Biochem Behav 1995;52:255–9.

Patel S, Roberts J, Moorman J, Reavill C. Localization of serotonin-4 receptors in the striatonigral pathway in the rat brain. Neuroscience 1995;69:1159–67.

Pellegrino LJ, Cushman AJ. A stereotaxic atlas of the rat brain. New York: Meredith, 1967.

Pires JGP, Ramage AG, Silva SR, Futuro-Neto HA. Effects of the 5-HT receptor antagonists cyanopindolol, ICI 169369, cisapride and granisetron on neuroleptic-induced catalepsy in mice. Braz J Med Biol Res 1993;26:847–52.

Pozzi L, Trabace L, Invernizzi R, Samanin R. Intranigral GR 113808, a selective 5-HT_4 receptor antagonist, attenuates morphine-stimulated dopamine release in the rat striatum. Brain Res 1995;692:265–8.

Reavill C, Hatcher JP, Lewis VA, Sanger GJ, Hagan J. 5-HT_4 receptors antagonism does not affect motor and reward mechanisms in the rat. Eur J Pharmacol 1998;357:115–20.

Sanberg PR. Haloperidol-induced catalepsy is mediated by postsynaptic dopamine receptors. Nature 1980;284:472–3.

Sempere AP, Duarte J, Cabezas C, Claveria LE, Coria F. Aggravation of parkinsonian tremor by cisapride. Clin Neurophamacol 1995;18:76–8.

Silvestre JS, Fernandez AG, Palacios JM. Effects of 5-HT4 receptor antagonists on rat behaviour in the elevated plus-maze test. Eur J Pharmacol 1996;309:219–22.

Soubrié P, Reisine TD, Glowinski J. Functional aspects of serotonin transmission in the basal ganglia: a review and in vivo approach using the push–pull cannula technique. Neuroscience 1984;13:605–25.

Spampinato U, Nowakowska E, Samanin R. Increased release of striatal dopamine after long-term treatment with methadone in rats: inhibition by agents which increase central 5-hydroxytryptamine transmission. J Pharm Pharmacol 1983;35:831–3.

Steward LJ, Ge J, Stowe RL, Brown DC, Bufton RK, Stokes PRA, Barnes NM. Ability of 5-HT_4 receptor ligands to modulate rat striatal dopamine release in vitro or in vivo. Br J Pharmacol 1996;117:55–62.

Taylor SG, Routledge C. Lack of effect of systemically administered 5-HT_4 agonists on dopamine levels measured from the nucleus accumbens and striatum: an in vivo microdialysis study in freely-moving rats. Br J Pharmacol 1996;117:326P (suppl.).

Themann P, Havemann U, Kuschinsky K. Development of tolerance to the muscular rigidity produced by morphine and replacement by stereotypic behaviour. Neuropeptides 1984;5:27–8.

Thorré K, Ebinger G, Michotte Y. 5-HT_4 receptor involvement in the serotonin-enhanced dopamine efflux from the substantia nigra of the freely moving rat: a microdialysis study. Brain Res 1998;796:117–24.

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.

Ward RP, Dorsa DM. Molecular and behavioral effects mediated by Gs-coupled adenosine A_{2a}, but not serotonin 5-HT_4 or 5-HT_6 receptors following antipsychotic administration. Neuroscience 1999;89:927–38.