European Journal of Pharmacology 634 (2010) 89–94

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Behavioural Pharmacology

Increase in locomotor activity after acute administration of the nicotinic receptor agonist 3-bromocytisine in rats

Juan Andrés Abin-Carriquiry [a,*,1], Jessika Urbanavicius [a,1], Cecilia Scorza [b], Marcos Rebolledo-Fuentes [c], Susan Wonnacott [d], Bruce K. Cassels [c], Federico Dajas [a]

[a] Department of Neurochemistry, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay
[b] Cell Biology Laboratory, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay
[c] Department of Chemistry, Faculty of Sciences, University of Chile, Santiago, Chile
[d] Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, UK

ARTICLE INFO

Article history:
Received 22 December 2009
Received in revised form 28 January 2010
Accepted 14 February 2010
Available online 23 February 2010

Keywords:
Locomotion
3-Bromocytisine
Dopamine
Nicotinic receptor

ABSTRACT

Nicotinic acetylcholine receptors influence striatal dopaminergic activity and its outcome on motor behavior. For these reasons, nicotinic receptors have been considered as therapeutically relevant targets for Parkinson's disease, in which a dramatic loss of dopamine affects motor functions. The aim of the present work was to compare the effects on locomotor activity induced by the nicotinic agonist cytisine and two brominated derivatives, 5- and 3-bromocytisine (5-BrCy and 3-BrCy) using nicotine for comparison. After acute systemic administration of the agonists only 3-BrCy induced an increase in locomotor activity. To study the mechanism of action involved in this increase we co-administered 3-BrCy with the nicotinic antagonist mecamylamine and also examined 3-BrCy's effects in rats pre-treated with the long acting nicotinic antagonist chlorisondamine, administered directly in the dorsal and ventral striatum. We studied the role of the dopaminergic system by co-administration of the D2 dopamine receptor antagonist, haloperidol. The results indicate that the increase in motor activity elicited by 3-BrCy was mediated by nicotinic receptors in the dorsal and ventral striatum and depends on the interaction of nicotinic receptors with the dopaminergic system. We conclude that 3-BrCy might be a new tool to study the modulation of the dopaminergic system by nicotinic receptors and their behavioral implications.

1. Introduction

A prominent role of nicotinic receptors in the central nervous system is modulation of neurotransmission through presynaptically-located heteroreceptors and the nicotinic modulation of dopamine release in the striatum has been well studied (Wonnacott, 1997). The dopaminergic system is a key element in the control of locomotion, dorsal and ventral striatum receive dopaminergic afferents from the substantia nigra and ventral tegmental area respectively (Maskos, 2008). Nicotinic receptors present in somatodendritic and terminal regions regulate cell excitability, dopamine release and neuronal integration influencing striatal outcome and eliciting different locomotor effects (Exley and Cragg, 2008; Livingstone and Wonnacott, 2009). Chronic administration of nicotine induces a marked increase in locomotor activity that is accompanied by increased dopamine metabolism, mainly in the mesolimbic pathway (Benwell and Balfour, 1992; King et al., 2004; Marubio et al., 2003). This behavioral effect contrasts with the initial locomotor depression described for acute nicotine (Vezina et al., 2007).

Because of the ability of nicotinic receptors to modulate the dopaminergic system, they have been proposed as therapeutically relevant targets for Parkinson's disease (Quik et al., 2010). Epidemiological studies have shown that smokers are at lower risk for Parkinson's disease than non-smokers and it has been hypothesized that nicotine is responsible for this effect (Tutka and Zatonski, 2006). In spite of these potential beneficial actions, the use of nicotine for therapy is limited by unwanted effects associated with ganglionic stimulation. In this sense, the development of nicotinic drugs with subtype selectivity and/or low addiction liability is a key issue in the quest for new compounds with potential therapeutic value (Cassels et al., 2005).

Cytisine is an alkaloid present in many plants of the legume family that shows higher affinity for heteromeric α4β2 nicotinic receptors than nicotine. In previous studies we have shown that halogen substitution at C3 of the cytisine structure led to increased binding affinity and *in vitro* functional potency for α4β2 and α7 nicotinic receptors, compared with cytisine. Halogen substitution at C5 resulted in a slight decrease in activity. 3-Bromocytisine (3-BrCy) and 5-bromocytisine (5-BrCy) show, respectively, increased or reduced potency to induce dopamine release

* Corresponding author. Department of Neurochemistry, Instituto de Investigaciones Biológicas Clemente Estable, Avenida Italia 3318, 11600. Montevideo, Uruguay. Tel.: +598 2 487 16 16x123/121; fax: +598 2 487 26 03.
E-mail address: abin@adinet.com.uy (J.A. Abin-Carriquiry).
[1] J.A.A-C. and J.U. contributed equally to this work.

0014-2999/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2010.02.030

from striatal slices compared with cytisine. When delivered by reverse microdialysis, 3-BrCy was more potent than cytisine and 5-BrCy at increasing extracellular dopamine levels *in vivo* although 3-BrCy showed lower efficacy than the other agonists (Abin-Carriquiry et al., 2008, 2006).

In the present study, we compared the effects of cytisine, 3-BrCy and 5-BrCy on motor behavior, following systemic administration in non-habituated rats, using nicotine as the well-established comparator. Previous studies have proposed that the effects of nicotinic receptor agonists on locomotion could be an indication of their potential as dopamine releasers and therapeutic candidates in Parkinson's disease (Bednar et al., 2004; Janhunen and Ahtee, 2004; Janhunen et al., 2005). Our results showed a striking and significant increase in locomotor activity induced by 3-BrCy when compared with nicotine, cytisine and 5-BrCy. This was mediated by nicotinic receptors in the dorsal and ventral striatum, and the dopaminergic system was implicated by using the dopamine receptor antagonist haloperidol.

2. Methods

2.1. Animals

Male Sprague Dawley rats (250–300 g) obtained from the IIBCE animal facilities (Montevideo) were used in this study. They were housed in groups of 6 in clear plastic cages (50×30×20 cm) with food and water *ad libitum* and kept under controlled conditions (temperature 22±2 °C, 12-h day–night cycle, lights on at 7:00 a.m.). The experiments were conducted according to ethical guidelines and the research project was approved by the Ethical Committee of the Clemente Estable Institute. Adequate measures were taken to minimize pain, discomfort or stress of the animals. In addition, efforts were made to use only the minimal number of animals necessary to produce reliable scientific data.

2.2. Drugs and reagents

(−)-Nicotine hydrogen tartrate, mecamylamine and sodium octyl sulphate were obtained from Sigma Chemical Co. (Poole, Dorset, UK) while haloperidol hydrochloride was from Tocris (Ellisville, MO, USA). Chemicals for HPLC analysis, and artificial cerebrospinal fluid were purchased from Mallinckrodt Baker. Ketamine (50 mg/ml) and xylazine (20 mg/ml) were from Konsol Köning (Buenos Aires, Argentina). Chlorisondamine was donated by Novartis Pharmaceuticals (East Hanover, NJ, USA).

Cytisine and its derivatives were obtained as previously described and used as the hydrochloride salts (Houlihan et al., 2001).

Nicotine, cytisine, 5-BrCy, 3-BrCy, mecamylamine and haloperidol were dissolved in saline and the pH was adjusted to 7.4. Aliquots were prepared and stored at −20 °C. Drugs were injected subcutaneously (s.c.) and control groups received the corresponding vehicle injection. Nicotine, cytisine and 5-BrCy were injected at 0.72 mg/kg; 3-BrCy at 0.05, 0.1 and 0.2 mg/kg, and mecamylamine at 3 mg/kg. Nicotinic agonist doses were chosen based on previous work (Abin-Carriquiry et al., 2008). In the case of 3-BrCy, a dose of 1 mg/kg induced tonic–clonic convulsions that lasted approximately 5 min. These effects limited the concentrations that could be evaluated *in vivo* (Abin-Carriquiry et al., 2008). Haloperidol was administered at 0.025 mg/kg. All drugs were administered in a volume of 1 ml/kg. Chlorisondamine was dissolved in artificial cerebrospinal fluid and injected intracerebrally.

2.3. Treatments

In experiments involving a single drug injection, nicotine, cytisine, 5-BrCy, 3-BrCy or saline was administered 5 min before starting the behavioral test (see Fig. 1A). In experiments assessing the systemic mecamylamine or haloperidol reversal of 3-BrCy actions, the antagonists were administered 10 min before starting the behavioral test (see Fig. 1A). In experiments assessing the local antagonism of 3-BrCy-induced behaviors, chlorisondamine was administered locally at 10 µg intra-dorsal striatum and 5 µg intra-accumbens 3 days before the behavioral tests (Fig. 1B).

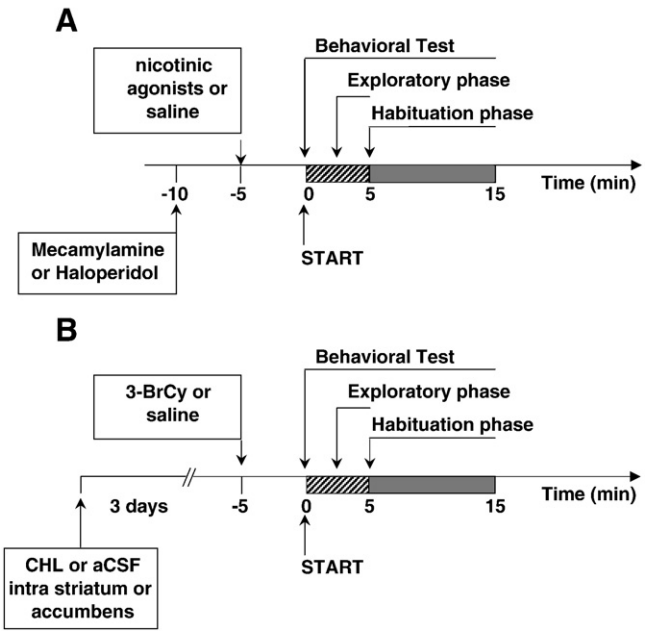

Fig. 1. Schematic representation of the experimental protocols. CHL: chlorisondamine; aCSF: artificial cerebrospinal fluid.

2.4. Behavioral experiments

Rats were brought to the experimental room in their home cages, identified and weighed one day before the behavioral experiments to allow acclimatization to the test environment. The experimental room was under controlled temperature (22±2 °C) and the behavioral testing was conducted using the open field paradigm. Briefly, the open field apparatus consists of a square Plexiglas cage (60×60×40 cm) with red walls to minimize outside light and noise. The open field is equipped with an optical system to measure animal activity, consisting of eight photocell beams on each side of the arena, forming a grid of 64 equally sized squares. The open field is connected to a computer running a Motor Behavioral Monitor (MBM) software which automatically records the number of beam crosses. Experiments were carried out under dim light.

On the day of the experiment, the animals were administered either drug or vehicle. After injection, rats were individually placed in the centre of the open field and were left to move freely during a 15 min period. Before placing each individual rat in the open field, the floor was covered with a thin layer of fresh gravel. After behavioral monitoring, the gravel was removed and the floor was cleaned with an alcohol:water solution and left to dry before testing the next rat. All animals were naïve to the open field.

Two different periods were distinguished and computed: a first 0–5 min period corresponding to the exploratory phase of the unfamiliar open field and a further 5–15 min period corresponding to the habituation phase (see Fig. 1A–B) (Scorza et al., 2008). In each phase the number of beam crosses and rearings (reflecting animal exploratory activity) was measured. Rearings were scored as the number of times that a rat reared up on its hindlimbs irrespective of whether the animal showed on- or off-wall rearing.

Behaviors were scored during the actual experiments by two experimenters, one of them blind to the treatment. Rats were used only once and experimental groups were balanced to keep all treatments constant in each experimental session. Ratings were

carried out simultaneously by two experimenters. All experiments were performed between 8:00 and 13:00.

2.5. Intracerebral injections of chlorisondamine

Animals were anesthetized with an intraperitoneal (i.p.) injection of a mixture of ketamine (90 mg/kg) and xylazine (5 mg/kg) and placed in a stereotaxic frame (David Kopf Instruments, USA). Following scalp incision, skull landmarks were visualized and coordinates were determined from bregma according to Paxinos (1986). A small hole was made in the skull at the injection site according to the selected coordinates. The duramater was punctured with a 25G needle and a stainless steel administration cannula (outer diameters 0.5 mm) was lowered bilaterally to reach the dorsal striatum (AP = +0.6 mm, L = ±3.2 mm). Two dorsoventral injections at −7.0 and −4.5 mm were made. In total, four injections were made in each rat (two per hemisphere). For accumbens, the administration cannula was lowered from bregma in AP = +1.7 mm, L = ±1.4 mm (bilateral) and DV = −6.4 mm. Two injections in each rat were made (one per hemisphere). Chlorisondamine or vehicle artificial cerebrospinal fluid (1 μg/μl) was injected through the administration cannula connected to a microsyringe pump with a flow rate of 2 μl/min, in two injections of 5 μl per hemisphere in the dorsal striatum (final dose 10 μg per side) and one injection for the accumbens (final dose 5 μg per side).

2.6. Data analysis

Data are given as Mean ± Standard Error of the Mean (S.E.M.). Kolmogorov–Smirnov normality tests were performed. One-way analysis of variance (ANOVA) followed by *post hoc* Newman–Keuls multiple comparison test were used to perform the data analysis. Statistical significance was set at $P<0.05$.

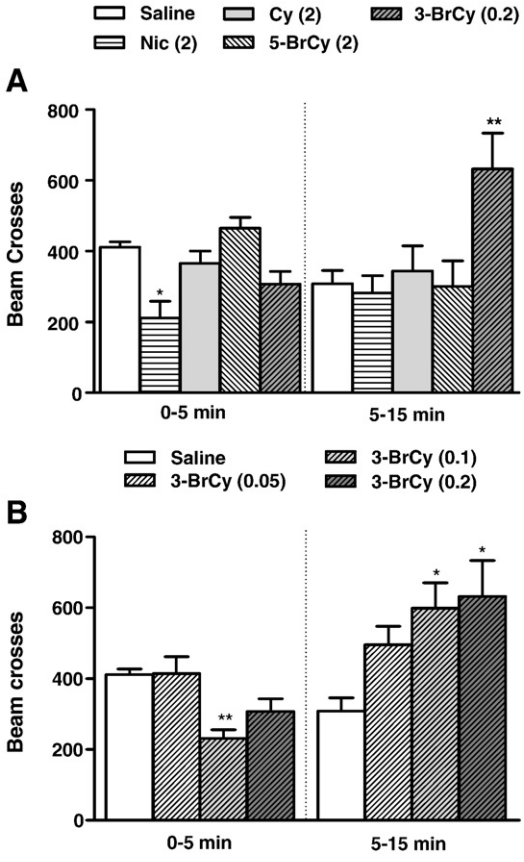

Fig. 2. A). Effects of systemic administration of nicotinic agonists on locomotor activity in rats naïve to the test environment, recorded in the OF during 15 min. B). Dose-related response to 3-BrCy in locomotor activity in the OF. Data are expressed as mean ± S.E.M. of the number of beam crosses in the 0–5 min and 5–15 min phases. * = *vs* Saline. One-way ANOVA followed by Newman–Keuls. * = $P<0.05$; ** = $P<0.01$. n = 6–8/group. Nic: nicotine; Cy: cytisine.

Table 1
Effects of nicotinic agonists on rearing behavior.

Treatment	Number of rearings (0–5 min)	Number of rearings (5–15 min)
Saline	28.0 ± 2.7	14.2 ± 2.2
Nicotine (2.0)	0.2 ± 0.1[b]	0.7 ± 0.2[a]
Cytisine (2.0)	22.2 ± 2.9	13.2 ± 3.1
5-BrCy (2.0)	23.0 ± 2.3	11.7 ± 3.7
3-BrCy (0.2)	1.3 ± 0.4[b]	1.6 ± 0.6[a]

Data are expressed as mean ± S.E.M. of number of rearings during 0–5 min and 5–15 min. One-way ANOVA followed by Newman–Keuls. [a,b] = *vs* Sal group; [a] = $P<0.01$; [b] = $P<0.001$. n = 6–8/group.

3. Results

3.1. Behavioral effects of systemic administration of nAChR agonists

Fig. 2A shows the results of locomotor activity induced by acute systemic administration of nicotinic agonists (cytisine, 3-BrCy, 5-BrCy and nicotine). During the exploratory phase (0–5 min) nicotine (0.72 mg/kg; as well as 0.36 mg/kg, data not shown) induced a significant decrease in locomotor activity ($P<0.05$), whereas neither cytisine (2 mg/kg) nor 3-BrCy (0.2 mg/kg) and 5-BrCy (2 mg/kg) altered locomotor activity. During the habituation phase (5–15 min), 3-BrCy (0.2 mg/kg) was the only nicotinic agonist that induced a significant increase in locomotor activity ($P<0.01$).

The dose response relationship for 3-BrCy showed that during the exploratory phase the 0.1 mg/kg dose induced a significant decrease of locomotor activity. However, during the habituation period there was a significant increase in locomotor activity with both 0.1 and 0.2 mg/kg 3-BrCy doses compared to saline ($P<0.05$; Fig. 2B).

Rearings were almost completely blocked by nicotine and 3-BrCy administration ($P<0.001$) in both 0–5 and 5–15 min periods while cytisine and 5-BrCy had no effect. The general demeanor of the animals was not changed by the drug treatments (Table 1).

3.2. Effect of systemic administration of mecamylamine on the motor behavior induced by 3-BrCy

The systemic co-administration of the non-competitive nicotinic antagonist mecamylamine completely blocked the hyperlocomotion induced by 3-BrCy ($P<0.01$) during the habituation phase, whereas mecamylamine did not affect basal locomotor activity (Fig. 3). During the exploratory phase there were no changes between treatments,

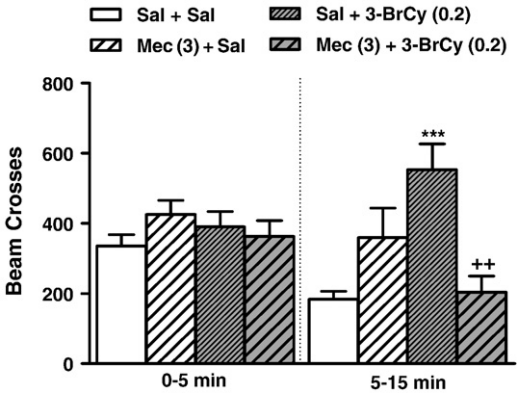

Fig. 3. Antagonism by mecamylamine of the locomotor activity induced by 3-BrCy. Data are expressed as mean ± S.E.M. of the number of beam crosses in the 0–5 min and 5–15 min phases. * = *vs* Sal + Sal; + = *vs* Sal + 3-BrCy. One-way ANOVA followed by Newman–Keuls. ++ = $P<0.01$; *** $P<0.001$. n = 6–8/group. Sal: saline.

Table 2
Effect of mecamylamine (Mec) or haloperidol (Hal) on the rearing behavior induced by 3-BrCy.

Treatment	Number of rearings(0–5 min)	Number of rearings (5–15 min)
Sal + Sal	27.1 ± 4.7	11.4 ± 2.7
Mec + Sal	25.5 ± 5.1	13.2 ± 1.9
Sal + 3-BrCy	4.1 ± 1.4[a]	1.0 ± 0.3[a]
Mec + 3-BrCy	18.3 ± 1.7[b]	4.8 ± 1.5
Hal + Sal	28.0 ± 5.5	15.5 ± 3.5
Hal + 3-BrCy	3.6 ± 1.4[c]	4.0 ± 1.8[d]

Data are expressed as mean ± S.E.M. of the number of rearings during 0–5 min and 5–15 min periods. [a] = *vs* Sal + Sal; [b] = *vs* Sal + 3-BrCy; [c,d] = Hal + Sal *vs* Hal + 3-BrCy. One-way ANOVA followed by Newman–Keuls. [b,d] = $P<0.01$; [a,c] = $P<0.001$. $n = 6$–8/group. Sal: saline.

consistent with the previous experiments using this dose of 3-BrCy (Figs. 1 and 2).

Co-administration of mecamylamine also prevented the decrease in the number of rearings induced by 3-BrCy ($P<0.01$) during the exploratory phase (Table 2). During the habituation phase mecamylamine did not reverse the significant decrease of rearings induced by 3-BrCy. The basal level of rearings following saline treatment was not altered by mecamylamine during either the exploratory or the habituation phase.

3.3. Effect of intra-striatal and intra-accumbens administration of chlorisondamine on the behavioral effect induced by 3-BrCy

In rats pre-treated with an intracerebral injection of chlorisondamine into the dorsal striatum, the increase in locomotor activity during the habituation period (5–15 min) in response to 3-BrCy (0.1 mg/kg) was completely prevented ($P<0.01$), without modification of spontaneous locomotor activity (Fig. 4A). During the initial exploratory period (0–5 min) there were no changes between treatments.

Administration of chlorisondamine into the accumbens totally blocked the hyperlocomotion elicited in 3-BrCy-treated rats ($P<0.01$) (Fig. 4B). As for intra-striatal chlorisondamine, intra-accumbens chlorisondamine did not change the spontaneous locomotor activity.

In the case of rearing behavior, intra-striatal or intra-accumbens injection of chlorisondamine significantly prevented the decrease in the number of rearings induced by 3-BrCy, without modifying spontaneous rearing activity during the exploratory phase (Table 3). Antagonism by chlorisondamine was only observed during the exploratory phase, since 3-BrCy did not change the number of rearings during the habituation phase.

Table 3
Effect of chlorisondamine (CHL) injection into the dorsal or ventral striatum on rearings induced by 3-BrCy.

Pre-treatment	Treatment	Number of rearings (0–5 min)	Number of rearings (5–15 min)
Intra-striatal			
aCSF	Saline	25.4 ± 3.3	11.2 ± 4.0
aCSF	3-BrCy (0.1)	7.2 ± 2.9 [a]	7.6 ± 2.6
CHL	Saline	25.6 ± 3.2	7.1 ± 2.0
CHL	3-BrCy (0.1)	26.5 ± 2.7[b]	7.8 ± 3.3
Intra-accumbens			
aCSF	Saline	23.0 ± 2.8	5.5 ± 2.1
aCSF	3-BrCy (0.1)	5.1 ± 2.0[a]	2.5 ± 0.9
CHL	Saline	19.1 ± 3.2	12.4 ± 2.3
CHL	3-BrCy (0.1)	19.2 ± 3.3[c]	8.2 ± 4.0

Data are expressed as mean ± S.E.M. of the number of rearings during 0–5 min and 5–15 min periods. [a] = *vs* aCFS + Sal; [b,c] = *vs* aCFS + 3-BrCy. One-way ANOVA followed by Newman–Keuls. [c] = $P<0.01$; [a,b] = $P<0.001$. $n = 8$/group. aCSF: artificial cerebrospinal fluid.

3.4. Effect of systemic administration of haloperidol on the behavioral effects induced by 3-BrCy

The co-administration of 3-BrCy and haloperidol induced a significant decrease in locomotor activity during the exploratory phase, compared with the response to either drug alone (Fig. 5). During the subsequent 5–15 min period, haloperidol at 0.025 mg/kg attenuated the hyperlocomotion induced by 3-BrCy ($P<0.05$) without affecting spontaneous locomotor activity (Fig. 5). Haloperidol did not modify the significant decrease in rearings induced by 3-BrCy, and had no effect on basal rearing activity (Table 2).

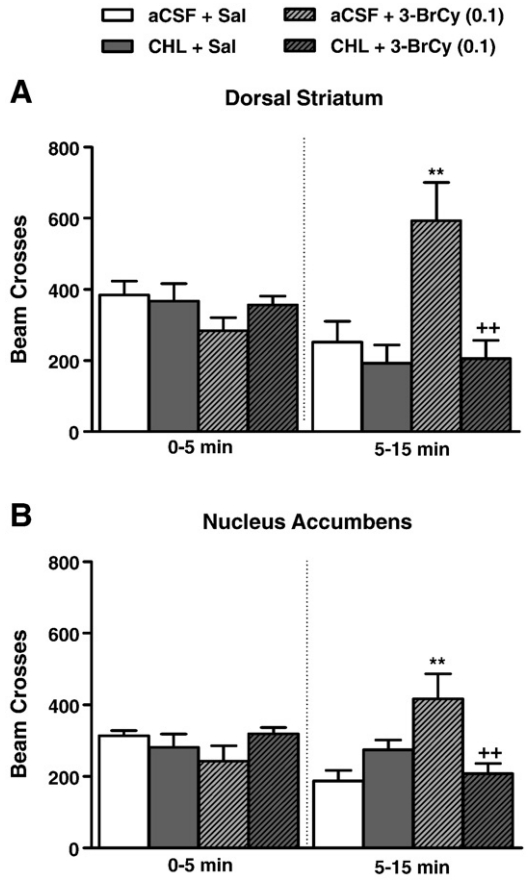

Fig. 4. Antagonism by chlorisondamine (CHL) injected into the dorsal (A) or ventral (B) striatum of the locomotor effects induced by 3-BrCy. Data are expressed as mean ± S.E.M. of the number of beam crosses during 0–5 min and 5–15 min periods. * = *vs* aCSF + sal aCFS; + = *vs* aCFS + 3-BrCy. One-way ANOVA followed by Newman–Keuls. **, ++ = $P<0.01$. $n = 8$/group. Sal: saline; aCSF: artificial cerebrospinal fluid.

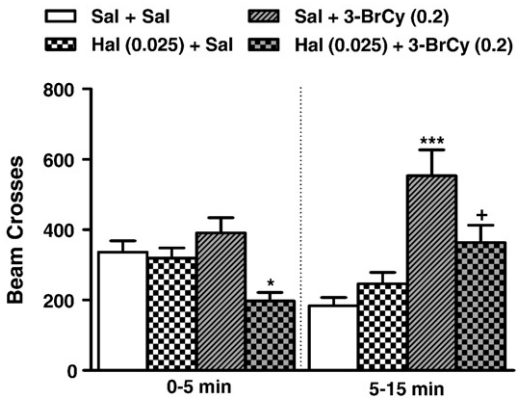

Fig. 5. Effect of haloperidol (Hal) on the locomotor activity induced by 3-BrCy. Data are expressed as mean ± S.E.M. of the number of beam crosses in the 0–5 min and 5–15 min phases. * = *vs* Sal + Sal; + = *vs* Sal + 3-BrCy. One-way ANOVA followed by Newman–Keuls. *, + = $P<0.05$; *** = $P<0.001$. $n = 8$/group. Sal: saline.

4. Discussion

The results described above show that, in contrast to nicotine, cytisine and 5-BrCy, acute systemic administration of 3-BrCy induced an increase in locomotor activity in naïve rats, revealing a novel and distinct profile for a nicotinic receptor agonist. Blockade by mecamylamine confirmed that the motor effects of 3-BrCy are mediated by nicotinic receptors.

Changes in locomotor activity that occur after the administration of nicotine and other nicotinic receptor agonists in rats have been extensively documented. An increase in locomotor activity occurs consistently after repeated nicotinic agonist administration followed by a subsequent challenge, a phenomenon known as behavioral sensitization (Menzaghi et al., 1997; Pawlak and Schwarting, 2005). The increase in locomotor activity during sensitization is recognized as a plastic event related to the modulation of dopamine release mainly in the mesolimbic pathway by β2 subunit-containing nicotinic receptors (Govind et al., 2009; King et al., 2004).

However, the acute effects of nicotinic agonists on locomotion can vary with sex, age and the environment, or previous exposure to the drug (Belluzzi et al., 2004; Benwell and Balfour, 1992; Menzaghi et al., 1997; Prus et al., 2008; Schochet et al., 2004). Previous work has shown that acute nicotine failed to induce increases in locomotor activity in animals not habituated to the environment and even caused an initial phase of locomotor depression and ataxia, effects associated with peripheral nicotinic receptor stimulation that undergo rapid tolerance (Clarke and Kumar, 1983; Stolerman et al., 1974). Consistent with this, we observed that nicotine (0.72 mg/kg) and 3-BrCy (0.1 mg/kg) significantly reduced locomotor activity during the initial exploratory phase but once animals were habituated to the test environment, only 3-BrCy induced a significant increase in locomotion. This increase in locomotor activity was dependent on nicotinic receptors activation and could be partially prevented by haloperidol administration, suggesting the involvement of dopamine release. This is compatible with our previous report that 3-BrCy locally applied into the dorsal striatum induces dopamine release (Abin-Carriquiry et al., 2008). Haloperidol was used at a low dose to avoid higher dopamine D2 receptor occupancy and interference with basal motor activity (Schotte et al., 1993). The differences observed between 3-BrCy and nicotine, 5-BrCy and cytisine could be related to their relative potencies and/or efficacies in eliciting dopamine release. Indeed, 3-BrCy is 10 times more potent than cytisine or nicotine in releasing striatal dopamine *in vitro* (Abin-Carriquiry et al., 2006) and *in vivo* (Abin-Carriquiry et al., 2008).

To establish whether nicotinic receptors expressed in the dorsal striatum and/or accumbens are predominantly involved in the motor effects of 3-BrCy we used chlorisondamine as a pharmacological tool. Chlorisondamine is a bisquaternary amine that produces a remarkably long-lasting blockade of the central response to nicotine (Clarke et al., 1994; el-Bizri and Clarke, 1994a,b). Local injection of chlorisondamine confirmed that both the striatum and accumbens are involved in the motor effects of 3-BrCy. Together with the results of haloperidol blockade, this suggests that dopaminergic transmission in both regions is participating in 3-BrCy effects through nicotinic receptors activation. Although the mesolimbic pathway is considered to have a major role in motivational behavior, this result is consistent with the observation that nicotine increases accumbens dopamine and locomotor activity by interacting with nicotinic receptors in this pathway (Goshima et al., 1996). However, we cannot rule out the involvement of other neurotransmitter systems in the acute locomotor effects of 3-BrCy.

In contrast to the increase in locomotor activity, 3-BrCy reduced rearing activity, an unusual effect for a drug that elicits hyperactivity. Rearing behavior is a component of the natural exploratory repertory and is directly related to environmental novelty (Bardo et al., 1990; Fink and Smith, 1980a,b; Geyer et al., 1986). It has been suggested that rearing behavior and locomotor activity do not necessarily reflect the same physiological mechanisms (Pawlak and Schwarting, 2002). In this case, 3-BrCy would appear to differentially affect these distinct types of motor behavior. In contrast, nicotine blocked rearing behavior without increasing locomotor activity during the habituation phase.

In the present work, we have demonstrated that the reduction in rearings elicited by 3-BrCy depends on activation of nicotinic receptors as it was prevented by systemic mecamylamine or by local injection of chlorisondamine. The differential effects of 3-BrCy on locomotor activation and rearings could be influenced by the involvement of different nicotinic receptor subtypes located in the striatum and accumbens (Exley et al., 2008).

5. Conclusions

Nicotinic receptors are therapeutically relevant targets for neurodegenerative diseases and smoking cessation therapy (Cassels et al., 2005; Quik et al., 2009; Tutka and Zatonski, 2006). Cytisine and varenicline (a cytisinoid analogue approved by the FDA in 2006) are in use for smoking cessation (Jimenez-Ruiz et al., 2009; Tutka, 2008). We previously reported that some cytisinoids can prevent dopamine loss in experimental Parkinsonism (Abin-Carriquiry et al., 2008).

Neuroprotective features together with the high potency and efficacy of 3-BrCy for increasing locomotor activity suggest its potential utility as a lead molecule for the development of new pharmacological tools for symptomatic treatment of Parkinson's disease.

Acknowledgements

This study was partially supported by Wellcome Trust Collaborative Initiative Grant 073295/Z/03/Z and PEDECIBA (Uruguay).

References

Abin-Carriquiry, J.A., Voutilainen, M.H., Barik, J., Cassels, B.K., Iturriaga-Vasquez, P., Bermudez, I., Durand, C., Dajas, F., Wonnacott, S., 2006. C3-halogenation of cytisine generates potent and efficacious nicotinic receptor agonists. Eur. J. Pharmacol. 536, 1–11.

Abin-Carriquiry, J.A., Costa, G., Urbanavicius, J., Cassels, B.K., Rebolledo-Fuentes, M., Wonnacott, S., Dajas, F., 2008. *In vivo* modulation of dopaminergic nigrostriatal pathways by cytisine derivatives: implications for Parkinson's Disease. Eur. J. Pharmacol. 589, 80–84.

Bardo, M.T., Bowling, S.L., Pierce, R.C., 1990. Changes in locomotion and dopamine neurotransmission following amphetamine, haloperidol, and exposure to novel environmental stimuli. Psychopharmacology (Berl.) 101, 338–343.

Bednar, I., Friberg, L., Nordberg, A., 2004. Modulation of dopamine release by the nicotinic agonist epibatidine in the frontal cortex and the nucleus accumbens of naive and chronic nicotine treated rats. Neurochem. Int. 45, 1049–1055.

Belluzzi, J.D., Lee, A.G., Oliff, H.S., Leslie, F.M., 2004. Age-dependent effects of nicotine on locomotor activity and conditioned place preference in rats. Psychopharmacology (Berl.) 174, 389–395.

Benwell, M.E., Balfour, D.J., 1992. The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity. Br. J. Pharmacol. 105, 849–856.

Cassels, B.K., Bermudez, I., Dajas, F., Abin-Carriquiry, J.A., Wonnacott, S., 2005. From ligand design to therapeutic efficacy: the challenge for nicotinic receptor research. Drug Discov. Today 10, 1657–1665.

Clarke, P.B., Kumar, R., 1983. The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. Br. J. Pharmacol. 78, 329–337.

Clarke, P.B., Chaudieu, I., el-Bizri, H., Boksa, P., Quik, M., Esplin, B.A., Capek, R., 1994. The pharmacology of the nicotinic antagonist, chlorisondamine, investigated in rat brain and autonomic ganglion. Br. J. Pharmacol. 111, 397–405.

el-Bizri, H., Clarke, P.B., 1994a. Blockade of nicotinic receptor-mediated release of dopamine from striatal synaptosomes by chlorisondamine administered *in vivo*. Br. J. Pharmacol. 111, 414–418.

el-Bizri, H., Clarke, P.B., 1994b. Blockade of nicotinic receptor-mediated release of dopamine from striatal synaptosomes by chlorisondamine and other nicotinic antagonists administered *in vitro*. Br. J. Pharmacol. 111, 406–413.

Exley, R., Cragg, S.J., 2008. Presynaptic nicotinic receptors: a dynamic and diverse cholinergic filter of striatal dopamine neurotransmission. Br. J. Pharmacol. 153 (Suppl 1), S283–S297.

Exley, R., Clements, M.A., Hartung, H., McIntosh, J.M., Cragg, S.J., 2008. Alpha6-containing nicotinic acetylcholine receptors dominate the nicotine control of dopamine neurotransmission in nucleus accumbens. Neuropsychopharmacology 33, 2158–2166.

Fink, J.S., Smith, G.P., 1980a. Mesolimbic and mesocortical dopaminergic neurons are necessary for normal exploratory behavior in rats. Neurosci. Lett. 17, 61–65.
Fink, J.S., Smith, G.P., 1980b. Mesolimbicocortical dopamine terminal fields are necessary for normal locomotor and investigatory exploration in rats. Brain Res. 199, 359–384.
Geyer, M.A., Russo, P.V., Masten, V.L., 1986. Multivariate assessment of locomotor behavior: pharmacological and behavioral analyses. Pharmacol. Biochem. Behav. 25, 277–288.
Goshima, Y., Miyamae, T., Nakamura, S., Miki, K., Kosaka, K., Misu, Y., 1996. Ventral tegmental injection of nicotine induces locomotor activity and L-DOPA release from nucleus accumbens. Eur. J. Pharmacol. 309, 229–233.
Govind, A.P., Vezina, P., Green, W.N., 2009. Nicotine-induced upregulation of nicotinic receptors: underlying mechanisms and relevance to nicotine addiction. Biochem. Pharmacol. 78, 756–765.
Houlihan, L.M., Slater, Y., Guerra, D.L., Peng, J.H., Kuo, Y.P., Lukas, R.J., Cassels, B.K., Bermudez, I., 2001. Activity of cytisine and its brominated isosteres on recombinant human alpha7, alpha4beta2 and alpha4beta4 nicotinic acetylcholine receptors. J. Neurochem. 78, 1029–1043.
Janhunen, S., Ahtee, L., 2004. Comparison of the effects of nicotine and epibatidine on the striatal extracellular dopamine. Eur. J. Pharmacol. 494, 167–177.
Janhunen, S., Tuominen, R.K., Piepponen, T.P., Ahtee, L., 2005. Nicotine and epibatidine alter differently nomifensine-elevated dopamine output in the rat dorsal and ventral striatum. Eur. J. Pharmacol. 511, 143–150.
Jimenez-Ruiz, C., Berlin, I., Hering, T., 2009. Varenicline: a novel pharmacotherapy for smoking cessation. Drugs 69, 1319–1338.
King, S.L., Caldarone, B.J., Picciotto, M.R., 2004. Beta2-subunit-containing nicotinic acetylcholine receptors are critical for dopamine-dependent locomotor activation following repeated nicotine administration. Neuropharmacology 47 (Suppl 1), 132–139.
Livingstone, P.D., Wonnacott, S., 2009. Nicotinic acetylcholine receptors and the ascending dopamine pathways. Biochem. Pharmacol. 78, 744–755.
Marubio, L.M., Gardier, A.M., Durier, S., David, D., Klink, R., Arroyo-Jimenez, M.M., McIntosh, J.M., Rossi, F., Champtiaux, N., Zoli, M., Changeux, J.P., 2003. Effects of nicotine in the dopaminergic system of mice lacking the alpha4 subunit of neuronal nicotinic acetylcholine receptors. Eur. J. Neurosci. 17, 1329–1337.
Maskos, U., 2008. The cholinergic mesopontine tegmentum is a relatively neglected nicotinic master modulator of the dopaminergic system: relevance to drugs of abuse and pathology. Br. J. Pharmacol. 153 (Suppl 1), S438–S445.
Menzaghi, F., Whelan, K.T., Risbrough, V.B., Rao, T.S., Lloyd, G.K., 1997. Effects of a novel cholinergic ion channel agonist SIB-1765F on locomotor activity in rats. J. Pharmacol. Exp. Ther. 280, 384–392.
Pawlak, C.R., Schwarting, R.K., 2002. Object preference and nicotine consumption in rats with high *vs*. low rearing activity in a novel open field. Pharmacol. Biochem. Behav. 73, 679–687.
Pawlak, C.R., Schwarting, R.K., 2005. Repeated nicotine treatment in rats with high versus low rearing activity: analyses of behavioural sensitisation and place preference. Psychopharmacology (Berl.) 178, 440–450.
Paxinos, G.A.C.W., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press.
Prus, A.J., Vann, R.E., Rosecrans, J.A., James, J.R., Pehrson, A.L., O'Connell, M.M., Philibin, S.D., Robinson, S.E., 2008. Acute nicotine reduces and repeated nicotine increases spontaneous activity in male and female Lewis rats. Pharmacol. Biochem. Behav. 91, 150–154.
Quik, M., Campos, C., Parameswaran, N., Langston, J.W., McIntosh, J.M., Yeluashvili, M., 2010. Chronic nicotine treatment increases nAChRs and microglial expression in monkey substantia nigra after nigrostriatal damage. J. Mol. Neurosci. 40 (1–2), 105–113.
Quik, M., Huang, L.Z., Parameswaran, N., Bordia, T., Campos, C., Perez, X.A., 2009. Multiple roles for nicotine in Parkinson's disease. Biochem. Pharmacol. 78, 677–685.
Schochet, T.L., Kelley, A.E., Landry, C.F., 2004. Differential behavioral effects of nicotine exposure in adolescent and adult rats. Psychopharmacology (Berl.) 175, 265–273.
Schotte, A., Janssen, P.F., Megens, A.A., Leysen, J.E., 1993. Occupancy of central neurotransmitter receptors by risperidone, clozapine and haloperidol, measured ex vivo by quantitative autoradiography. Brain Res. 631, 191–202.
Scorza, M.C., Meikle, M.N., Hill, X.L., Richeri, A., Lorenzo, D., Artigas, F., 2008. Prefrontal cortex lesions cause only minor effects on the hyperlocomotion induced by MK-801 and its reversal by clozapine. Int. J. Neuropsychopharmacol. 11, 519–532.
Stolerman, I.P., Bunker, P., Jarvik, M.E., 1974. Nicotine tolerance in rats; role of dose and dose interval. Psychopharmacologia 34, 317–324.
Tutka, P., 2008. Nicotinic receptor partial agonists as novel compounds for the treatment of smoking cessation. Expert Opin. Investig. Drugs 17, 1473–1485.
Tutka, P., Zatonski, W., 2006. Cytisine for the treatment of nicotine addiction: from a molecule to therapeutic efficacy. Pharmacol. Rep. 58, 777–798.
Vezina, P., McGehee, D.S., Green, W.N., 2007. Exposure to nicotine and sensitization of nicotine-induced behaviors. Prog. Neuropsychopharmacol. Biol. Psychiatry 31, 1625–1638.
Wonnacott, S., 1997. Presynaptic nicotinic ACh receptors. Trends Neurosci. 20, 92–98.