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Serotonergic neurotransmission mediates hypothermia induced by the *N*-phenylpiperazine antipsychotic prototypes LASSBio-579 and LASSBio-581

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Abstract

Previous studies have demonstrated that LASSBio-579 and LASSBio-581, two *N*-phenylpiperazine derivatives designed for the treatment of schizophrenia, are presynaptic dopamine D_2 receptor agonists that induce a hypothermic effect in mice that is not mediated by dopamine receptor activation. The aim of the present study was to investigate possible serotonergic mechanisms underlying hypothermia induced by LASSBio-579 and LASSBio-581 in CF1 mice. The reduction in core temperature was dose-dependent (15–60 mg/kg, i.p.) and occurred by the oral route (30 mg/kg). Pretreatment with haloperidol (4 mg/kg, i.p.) resulted in a synergistic hypothermic effect. Pretreatment with (±)DOI (0.25 mg/kg, i.p.), a serotonin 5-$HT_{2A/C}$ receptor agonist, reduced the hypothermic effect induced by LASSBio-579 and LASSBio-581 at 15 and 30 mg/kg, i.p. In contrast, (±)DOI enhanced the hypothermia induced by both compounds at 60 mg/kg, i.p. The serotonin 5-HT_{1A} antagonist WAY 100635 (0.05 mg/kg, s.c.) abolished the hypothermia induced by LASSBio-579 and diminished the hypothermia induced by LASSBio-581. Pretreatment with LASSBio579 (30 and 60 mg/kg, i.p.) and LASSBio-581 (60 mg/kg, i.p.) reduced the number of head-twitches induced by (±)DOI (2.5 mg/kg, i.p.). The ear-scratch response induced by (±)DOI was inhibited by both LASSBio-579 and LASSBio-581 at 60 mg/kg, i.p. These results indicate that LASSBio-579 and LASSBio-581 have mechanisms of action through the serotonergic neurotransmitter system.

Keywords: Schizophrenia; Antipsychotics; Hypothermia; LASSBio-579; LASSBio-581; *N*-phenylpiperazine derivatives

1. Introduction

Schizophrenia is a chronic psychiatric disorder that affects approximately 1% of the world's population. Diagnostic features of schizophrenia include positive symptoms (e.g., auditory hallucinations, disorganized thoughts, delusions), negative symptoms (e.g., social withdrawal, avolition, anhedonia), and cognitive dysfunction (e.g., attentional impairment, memory deficits) (Wong and Van Tol, 2003; Marek and Merchant, 2005; Garzya et al., 2007). Schizophrenia conventionally has been treated with dopamine D_2 receptor antagonists such as haloperidol. However, these drugs have little effects on negative and cognitive symptoms and elicit extrapyramidal side effects at therapeutic doses (Gardner et al., 2005).

The introduction of clozapine for treatment-resistant schizophrenia gave rise to a new group of atypical antipsychotics. These drugs exhibit potent antagonism at multiple receptor subtypes, including dopamine and serotonin receptors (Meltzer, 1995; Farah, 2005; Gardner et al., 2005). However, a significant population of patients is still refractory to treatment, and these new drugs also induce serious side effects (Owens, 1996; Hirose et al., 2004; Farah, 2005), thus underscoring the importance of developing more effective and safer antipsychotic drugs.

With the goal of developing new atypical antipsychotics, a new series of *N*-phenylpiperazine derivatives was designed from

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hybridization of the lead compounds clozapine and L-741 (3-[(4-[4-chlorophenyl]piperazin-1-yl)methyl]-1*H*-pyrrolo[2,3]pyridine) (Menegatti et al., 2003). From this series, two derivatives [LASSBio-579: (1-[1-(4-chlorophenyl)-1*H*-4-pyrazolylmethyl]-4-phenylhexahydropyrazine); LASSBio-581: (1-[1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-ylmethyl]-4-phenylhexahydropyrazine)] were chosen for pharmacological studies. LASSBio-579 and LASSBio-581 displace YM-09151-2 [*cis*-*N*-(1-benzyl-2-methylpyrrolidine-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide] binding to D_2 receptors (IC_{50}=0.3 μM and 0.6 μM, respectively) but do not alter SCH 23390 (R-[+]-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) binding to D_1 receptors (Menegatti et al., 2003). Electrophysiological assays demonstrated that these derivatives act as agonists at presynaptic dopamine D_2 receptors (Menegatti et al., 2003). LASSBio-579 and LASSBio-581 were active in some tests predictive of typical antipsychotic activity. Both compounds caused discrete catalepsy in mice. LASSBio-579 demonstrated an inhibitory effect on amphetamine-induced stereotypy in rats and partially reversed apomorphine-induced hypothermia in mice (Neves et al., 2003). Unexpectedly, these derivatives also caused a reduction in core temperature in mice (Neves et al., 2003) that was not blocked by haloperidol (0.5 mg/kg, i.p.) (Neves et al., 2003). Taken together, these results indicate that hypothermia induced by LASSBio-579 and LASSBio-581 is not mediated by direct dopamine D_2 receptor activation and thus point to other neurotransmitter systems involved in this action.

Prior studies indicate that serotonin plays a key role in both central and peripheral mechanisms of thermoregulation (Schwartz et al., 1995). Hypothermia induced by serotonin 5-HT_{1A} receptor activation is a well recognized phenomenon (Goodwin et al., 1985; Forster et al., 1995). Atypical antipsychotics, such as clozapine and risperidone, produce a reduction in rodent corporal temperature that has been associated with 5-HT_2 receptor blockade (Ninan and Kulkarni, 1999; Oerther and Ahlenius, 2000). The development of antipsychotic drugs that modify both dopamine and serotonin receptors may be one approach to pharmacologically treat schizophrenia (Alvarado et al., 2005; Park et al., 2005; Brea et al., 2006; Garzya et al., 2007, Marek, 2007). The present study investigated the involvement of the serotonergic system in the effects of LASSBio-579 and LASSBio-581 on core body temperature in mice.

2. Materials and methods

2.1. Animals

Adult male CF1 mice (25–35 g) from the Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS-RS) breeding colony were used. The animals were housed in groups of 8–10 per cage (17×28×13 cm) with free access to food (Nuvital®) and water. The animals were kept at constant room temperature (22±1 °C) and humidity (60%) under a 12 h light–dark cycle (lights off at 7:00 pm). Mice were adapted to the housing conditions for at least 72 h before experimentation. All experiments were approved by CONEP — Brazil (National Commission of Research Ethics — Protocol 2006541) and performed according to National Research Ethical Committee guidelines (published by National Heath Council — MS, 1998), which are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Drugs and treatments

LASSBio-579 and LASSBio-581 were synthesized, purified, and structurally characterized as previously described (Menegatti et al., 2003). Haloperidol was purchased from Galena (São Paulo, SP, Brazil). (±)DOI hydrochloride and WAY 100635 maleate [*N*-(2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl)-*N*-(2-pyridinyl) cyclohexanecarboxamide maleate] were purchased from Sigma (São Paulo, SP, Brazil). LASSBio-579, LASSBio-581, and haloperidol were suspended in saline with an additional 1% (v/v) polissorbate 80. (±)DOI and WAY 100635 were directly dissolved in saline. Vehicle groups received 1% (v/v) polissorbate 80 in saline. The drugs were orally (p.o.) and intraperitonealy (i.p.) administered in a volume of 10 ml/kg and subcutaneously (s.c.) administered in a volume of 5 ml/kg. All doses are expressed as free base. Doses were chosen based on preliminary studies performed in our laboratory and on prior data (Forster et al., 1995; Yamada et al., 1995; Salmi and Ahlenius, 1998). Because the ultimate objective of our research is to develop lead compounds, the effects were investigated by two administration routes (Wermuth, 2006). Therefore, core temperature was evaluated by both oral and intraperitoneal routes. The oral dose was selected based on the intraperitoneal results.

2.3. Core temperature measurement

Core temperature was recorded with a digital thermometer (ProCheck) with a reading precision of 0.1 °C. Mice were gently immobilized. The thermometer was lubricated with Vaseline and inserted 1.5 cm into the animal's rectum. Basal temperature was recorded before the first treatment administration. Temperature measurements were performed between 9:00 a.m. and 11:00 a.m. at controlled room temperature (24±1 °C).

2.4. Influence of haloperidol, (±)DOI, and WAY 100635 on hypothermia induced by intraperitoneal administration of LASSBio-579 and LASSBio-581

Mice were pretreated with one of the challenge drugs (haloperidol, 4.0 mg/kg, i.p.; (±)DOI, 0.25 mg/kg, i.p.; WAY 100635, 0.05 mg/kg, s.c.) or vehicle (i.p.). Thirty minutes later, all animals received a second treatment with the test drugs LASSBio-579 or LASSBio-581 (15–60 mg/kg, i.p.). The body temperature of each animal was taken immediately prior to (basal temperature) and 45 and 60 min after pretreatment (i.e., 15 and 30 min after the second treatment).

2.5. Effect of oral LASSBio-579 and LASSBio-581 on core temperature

Each animal had its basal temperature measured and was immediately treated with vehicle, LASSBio-579 (30 mg/kg, p.o.),

or LASSBio-581 (30 mg/kg, p.o.). Core temperature was measured again 30 and 60 min after treatment.

2.6. Effect of LASSBio-579 and LASSBio-581 on head-twitches and ear-scratches induced by (±)DOI

This experimental protocol was adapted from Darmani et al. (1996). Animals were pretreated with LASSBio-579 or LASSBio-581 (15–60 mg/kg, i.p.) and placed in cylindrical glass cages (14 cm diameter, 15 cm high). Sixty minutes later, they were treated with (±)DOI (2.5 mg/kg, i.p.) or vehicle (posttreatment), and the number of head-twitches and ear-scratches was recorded for 30 min by two independent observers blind to treatment.

2.7. Statistical analysis

All temperature measurements were analyzed by two-way repeated-measures analysis of variance (ANOVA), with *Treatment* as the first factor and *Time Interval* of temperature measurement (second factor) as the repeated-measure. The number of head-twitches and ear-scratches was subjected to a one-way ANOVA. When necessary, Student–Newman–Keuls *post hoc* test was performed. Data were considered statistically significant at $P<0.05$. All analyses were performed using SigmaStat 2.03 software (Jandel Scientific Corporation).

3. Results

3.1. Influence of haloperidol, (±)DOI, and WAY 100635 on hypothermia induced by intraperitoneal administration of LASSBio-579 and LASSBio-581

The hypothermic effect of LASSBio-579 is shown in Fig. 1. LASSBio-579 induced dose-dependent hypothermia in mice, with no effect at 15 mg/kg, i.p., and a maximal effect at 30 mg/kg, i.p., 60 min after treatment (two-way repeated-measures ANOVA; *Treatment*, $F(3,167)=7.853$, $P<0.001$; *Time Interval*, $F(2,167)=35.590$, $P<0.001$; *Treatment* × *Time Interval* interaction, $F(6,167)=6.794$, $P<0.001$) (Fig. 1A). There was no difference in basal temperature among groups.

A single treatment with haloperidol (4.0 mg/kg, i.p.) induced a reduction in core temperature at 60 min. Coadministration of LASSBio-579 and haloperidol induced significant hypothermia (two-way repeated-measures ANOVA; *Treatment*, $F(4,197)=12.620$, $P<0.001$; *Time Interval*, $F(2,197)=82.334$, $P<0.001$; *Treatment* × *Time Interval* interaction, $F(8,197)=10.997$, $P<0.001$) (Fig. 1B). By comparing Fig. 1A and B, one can observe that haloperidol acted synergistically with LASSBio-579 to reduce body temperature. The hypothermic effect was more noticeable with 15 mg/kg LASSBio-579 but also was evident at higher doses (two-way repeated-measures ANOVA;

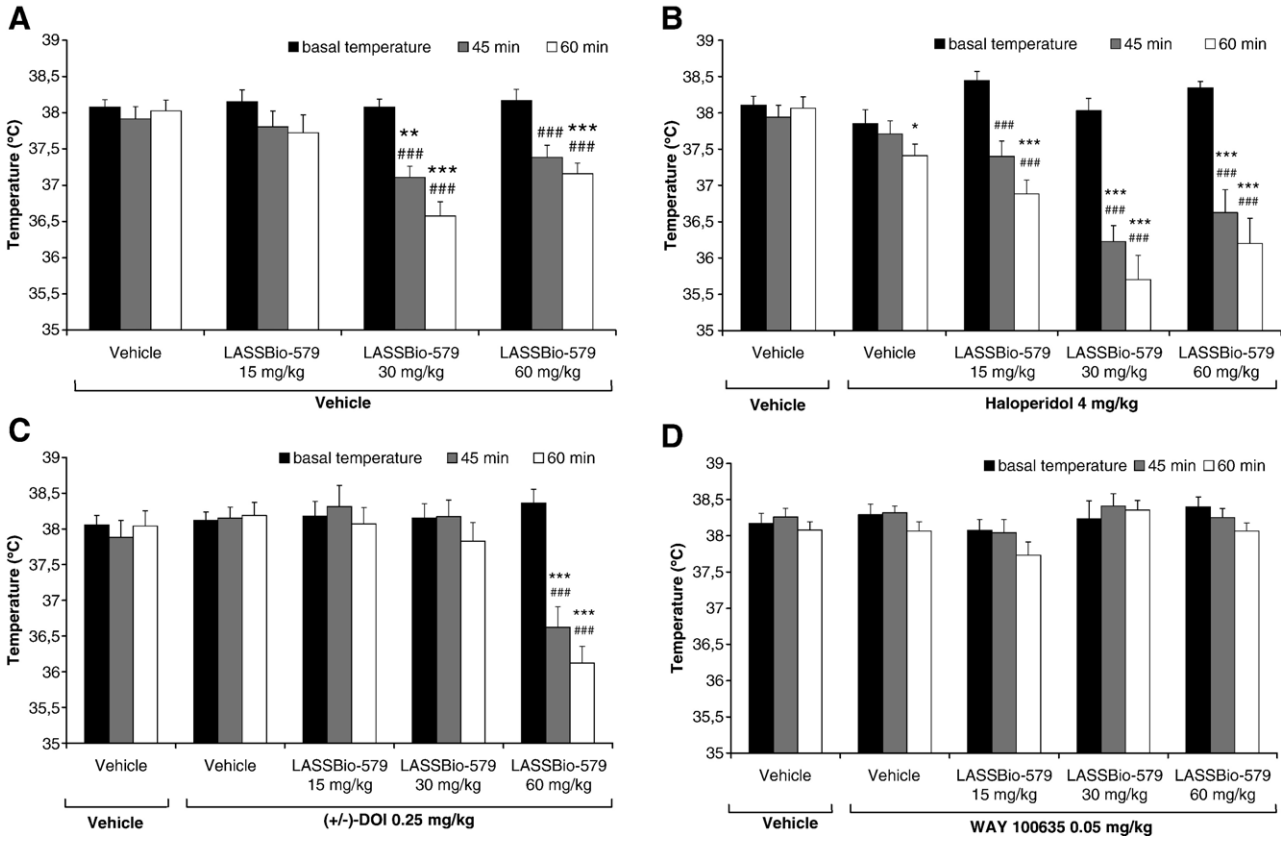

Fig. 1. Dose-response curve (A) and influence of haloperidol (B), (±)DOI (C), and WAY 100635 (D) pretreatment on the hypothermic effect of LASSBio-579 ($n=10$–16). Vehicle, LASSBio-579, haloperidol, and (±)DOI were administered intraperitoneally, and WAY 100635 was administered subcutaneously. Data are expressed as mean + S.E.M. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, different from vehicle + vehicle group at the same time of measurement; Student–Newman–Keuls *post hoc* test. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, different from basal temperature with the same treatment; Student–Newman–Keuls *post hoc* test.

Treatment, $F(7,320)=9.995$, $P<0.001$; *Time Interval*, $F(2,320)=121.555$, $P<0.001$; *Treatment*×*Time Interval* interaction, $F(14,320)=8.397$, $P<0.001$).

With regard to (±)DOI (0.25 mg/kg, i.p.), only cotreatment with 60 mg/kg LASSBio579 modified core temperature (two-way repeated-measures ANOVA; *Treatment*, $F(4,149)=8.392$, $P<0.001$; *Time Interval*, $F(2,149)=11.956$, $P<0.001$; *Treatment*×*Time Interval* interaction, $F(8,149)=9.200$, $P<0.001$) (Fig. 1C). The comparison with data in Fig. 1A revealed that (±)DOI prevented the hypothermic effect induced by 30 mg/kg LASSBio579. However, the hypothermic effect induced by 60 mg/kg LASSBio579 was increased by (±)DOI pretreatment (two-way repeated-measures ANOVA; *Treatment*, $F(7,269)=8.137$, $P<0.001$; *Time Interval*, $F(2,269)=40.114$, $P<0.001$; *Treatment*×*Time Interval* interaction, $F(14,269)=7.499$, $P<0.001$). Fig. 1D shows that cotreatment of WAY 100635 with LASSBio-579 did not change core temperature (two-way repeated-measures ANOVA; *Treatment*, $F(4,185)=1.389$, $P=0.249$; *Time Interval*, $F(2,185)=5.012$, $P=0.008$; *Treatment*×*Time Interval* interaction, $F(8,185)=0.835$, $P=0.574$), demonstrating that WAY 100635 (0.05 mg/kg, s.c.) completely abolished the hypothermic effect of LASSBio-579 (30 and 60 mg/kg) (two-way repeated-measures ANOVA; *Treatment*, $F(7,275)=10.300$, $P<0.001$; *Time Interval*, $F(2,275)=33.342$, $P<0.001$; *Treatment*×*Time Interval* interaction, $F(14,275)=5.670$, $P<0.001$) (Fig. 1A).

LASSBio-581 data are shown in Fig. 2. This *N*-phenylpiperazine derivative induced dose-dependent hypothermia in mice, with the maximal effect achieved at 30 mg/kg, i.p., and maintained at 60 mg/kg, i.p. There was no difference in basal temperature among groups (two-way repeated-measures ANOVA; *Treatment*, $F(3,158)=21.430$, $P<0.001$; *Time Interval*, $F(2,158)=127.792$, $P<0.001$; *Treatment*×*Time Interval* interaction, $F(6,158)=18.797$, $P<0.001$) (Fig. 2A).

All animals treated with haloperidol (4 mg/kg, i.p.) and LASSBio-581 exhibited a reduction in core temperature, and haloperidol induced a hypothermic effect at 60 min (two-way repeated-measures ANOVA; *Treatment*, $F(4,203)=18.724$, $P<0.001$; *Time Interval*, $F(2,203)=101.437$, $P<0.001$; *Treatment*×*Time Interval* interaction, $F(8,203)=15.238$, $P<0.001$) (Fig. 2B). When these data are compared to Fig. 2A, it is evident that haloperidol did not prevent the hypothermia induced by LASSBio-581. Conversely, it is remarkable that haloperidol administration caused a modest augmentation of the hypothermic effect induced by 60 mg/kg LASSBio-581 60 min after administration (two-way repeated-measures ANOVA; *Treatment*, $F(7,314)=15.139$, $P<0.001$;

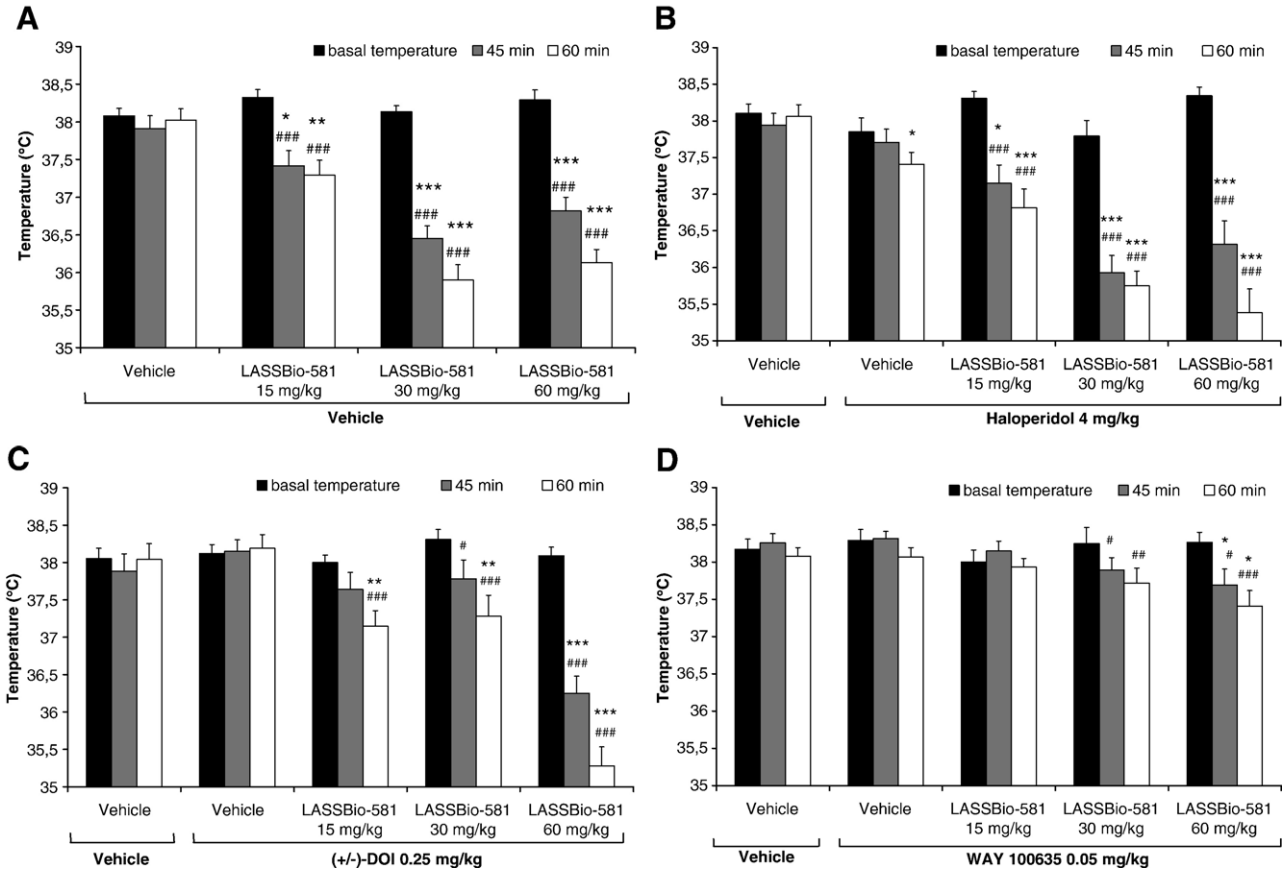

Fig. 2. Dose-response curve (A) and influence of haloperidol (B), (±)DOI (C), and WAY 100635 (D) pretreatment on the hypothermic effect of LASSBio-581 ($n=10$–16). Vehicle, LASSBio-581, haloperidol, and (±)DOI were administered intraperitoneally, and WAY 100635 was administered subcutaneously. Data are expressed as mean+S.E.M. *$P<0.05$, **$P<0.01$, ***$P<0.001$, different from vehicle+vehicle group at the same time of measurement; Student–Newman–Keuls *post hoc* test. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, different from basal temperature with the same treatment; Student–Newman–Keuls *post hoc* test.

Time Interval, F(2,314)=222.692, $P<0.001$; *Treatment × interaction, F(14,314)=12.160, P<0.001).*

LASSBio-581 caused a reduction in core temperature even with (±)DOI pretreatment (0.25 mg/kg, i.p.) (two-way repeated-measures ANOVA; *Treatment*, $F(4,149)=16.384$, $P<0.001$; *Time Interval*, $F(2,149)=51.790$, $P<0.001$; *Treatment × Time Interval* interaction, $F(8,149)=16.558$, $P<0.001$) (Fig. 2C). The comparison with data in Fig. 2A revealed that pretreatment with (±)DOI reduced the hypothermic effect induced by 15 and 30 mg/kg LASSBio-581 and increased the effect at a higher dose (60 mg/kg) (two-way repeated-measures ANOVA; *Treatment*, $F(7,260)=16.028$, $P<0.001$; *Time Interval*, $F(2,260)=166.065$, $P<0.001$; *Treatment × Time Interval* interaction, $F(14,260)=14.582$, $P<0.001$).

Results from the effect of WAY 100635 (0.05 mg/kg, s.c.) are shown in Fig. 2D. LASSBio-581 at 30 and 60 mg/kg elicited a hypothermic effect after WAY 100635 pretreatment (two-way repeated-measures ANOVA; *Treatment*, $F(4,188)=2.210$, $P=0.079$; *Time Interval*, $F(2,188)=10.293$, $P<0.001$; *Treatment × Time Interval* interaction, $F(8,188)=2.277$, $P=0.027$). WAY 100635 completely abolished the hypothermic effect induced by 15 mg/kg LASSBio-581 and markedly reduced hypothermia induced by 30 and 60 mg/kg LASSBio-581 (two-way repeated-measures ANOVA; *Treatment*, $F(5,278)=14.147$, $P<0.001$; *Time Interval*, $F(2,278)=91.703$, $P<0.001$; *Treatment × Time Interval* interaction, $F(14,378)=11.528$, $P<0.001$).

3.2. Effect of oral LASSBio-579 and LASSBio-581 on core temperature

Both LASSBio-579 and LASSBio-581 significantly reduced core temperature at 30 mg/kg, p.o. There was no difference in basal temperature between groups (two-way repeated-measures ANOVA; *Treatment*, $F(2,86)=1.889$, $P=0.171$; *Time Interval*, $F(2,86)=11.062$, $P<0.001$; *Treatment × Time Interval* interaction, $F(4,86)=4.512$, $P=0.003$) (Fig. 3). LASSBio-581 induced a slightly greater effect than LASSBio-579, especially at the second temperature measurement 30 min after drug administration.

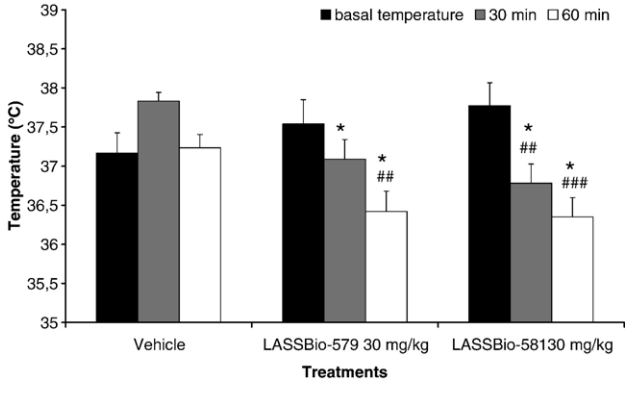

Fig. 3. Effect of LASSBio–579 (30 mg/kg, p.o.; $n=10$) and LASSBio-581 (30 mg/kg, p.o., $n=10$) on core temperature in mice. Vehicle group ($n=12$). Data are expressed as mean+S.E.M. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, different from vehicle+vehicle group at the same time of measurement; Student–Newman–Keuls *post hoc* test. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, different from basal temperature with the same treatment; Student–Newman–Keuls *post hoc* test.

Table 1
Effect of LASSBio-579 on head-twitches and ear-scratches induced by (±)DOI (2.5 mg/kg)

Pretreatment	Posttreatment	Head-twitches	Ear-scratches
Vehicle ($n=10$)	Vehicle	1.5±0.4	10.0±2.2
LASSBio-579			
15 mg/kg ($n=10$)	Vehicle	1.6±0.5$^{\#\#\#}$	9.6±1.9$^{\#\#\#}$
30 mg/kg ($n=10$)	Vehicle	1.7±0.6$^{\#\#\#}$	9.7±2.2$^{\#\#\#}$
60 mg/kg ($n=10$)	Vehicle	0.8±0.5$^{\#\#\#}$	7.4±2.2$^{\#\#\#}$
Vehicle ($n=9$)	(±)DOI 2.5 mg/kg	22.6±3.3***	101.1±17.2***
LASSBio-579			
15 mg/kg ($n=10$)	(±)DOI 2.5 mg/kg	17.5±3.2***	120.9±27.6***
30 mg/kg ($n=10$)	(±)DOI 2.5 mg/kg	15.5±1.9*** $^{\#}$	82.6±18.9**
60 mg/kg ($n=10$)	(±)DOI 2.5 mg/kg	10.9±1.7** $^{\#\#\#}$	43.1±9.3$^{\#\#}$

All treatments were administered intraperitoneally. Data are expressed as mean± S.E.M. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, different from vehicle+vehicle group in the Student–Newman–Keuls *post hoc* test.
$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, different from (±)DOI+vehicle group in the Student–Newman–Keuls *post hoc* test.

3.3. Effect of LASSBio-579 and LASSBio-581 on head-twitches and ear-scratches induced by (±)DOI

Pretreatment with 60 mg/kg LASSBio-581 significantly reduced the number of head-twitches (one-way ANOVA: $F(7,85)=17.728$, $P<0.001$) and ear-scratches (one-way ANOVA: $F(7,85)=9.384$, $P<0.001$) induced by 2.5 mg/kg (±)DOI, i.p. (Tables 1 and 2). LASSBio-579 at 60 mg/kg also reduced ear-scratches (one-way ANOVA: $F(7,86)=19.227$, $P<0.001$) and was more potent than LASSBio-581 at inhibiting head-twitches, eliciting the effect at 30 mg/kg (one-way ANOVA: $F(7,86)=11.400$, $P<0.001$).

Table 2
Effect of LASSBio-581 on head-twitches and ear-scratches induced by (±)DOI (2.5 mg/kg)

Pretreatment	Posttreatment	Head-twitches	Ear-scratches
Vehicle ($n=10$)	Vehicle	1.5±0.4	10.0±2.2
LASSBio-581			
15 mg/kg ($n=10$)	Vehicle	2.8±1.4$^{\#\#\#}$	10.4±2.3$^{\#\#\#}$
30 mg/kg ($n=10$)	Vehicle	1.6±0.5$^{\#\#\#}$	7.6±2.1$^{\#\#\#}$
60 mg/kg ($n=10$)	Vehicle	0.5±0.4$^{\#\#\#}$	3.6±1.4$^{\#\#\#}$
Vehicle ($n=9$)	(±)DOI 2.5 mg/kg	22.6±3.3***	101.1±17.2***
LASSBio-581			
15 mg/kg ($n=10$)	(±)DOI 2.5 mg/kg	19.9±2.2***	117.5±28.9***
30 mg/kg ($n=10$)	(±)DOI 2.5 mg/kg	20.7±3.5***	84.3±20.8**
60 mg/kg ($n=10$)	(±)DOI 2.5 mg/kg	14.0±2.5*** $^{\#}$	43.6±12.6$^{\#}$

All treatments were administered intraperitoneally. Data are expressed as mean± S.E.M. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, different from vehicle+vehicle group in the Student–Newman–Keuls *post hoc* test.
$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, different from DOI+vehicle group in the Student–Newman–Keuls *post hoc* test.

4. Discussion

Previous work demonstrated that LASSBio-579 and LASSBio-581 (30 mg/kg, i.p.) induced a reduction in core temperature in mice that was not prevented by haloperidol administration (0.5 mg/kg), thus indicating that the hypothermic effect was not directly mediated by dopamine neurotransmission (Neves et al., 2003). In the present study, we demonstrated that hypothermia also occurred, though by a lesser magnitude, when the compounds were administered orally (Fig. 3). This is an important finding because oral bioavailability is a crucial goal when searching for lead compounds for further drug development. Both derivatives work dose-dependently by the intraperitoneal route (Figs. 1A and 2A), but the hypothermic effect of LASSBio-581 is more pronounced at the doses tested. These findings are in agreement with the pharmacokinetic profiles of each compound. Both drugs showed bioavailability that was lower by the oral route than by the intraperitoneal route, and LASSBio-581 has greater bioavailability than LASSBio-579 (Tasso et al., 2005; Conrado, 2006).

Haloperidol (4 mg/kg, i.p.) elicited a hypothermic effect (Figs. 1B and 2B) as previously demonstrated (Yamada et al., 1995). Coadministration of LASSBio-579 or LASSBio-581 with haloperidol resulted in a synergistic hypothermic effect, especially with LASSBio-579 (Figs. 1 and 2). This experiment confirms our previous observation (Neves et al., 2003) that LASSBio-579 and LASSBio-581 hypothermic effects are not attributable to direct activation of dopamine D_2 postsynaptic receptors and points to the participation of other neurotransmitter systems in the effects of *N*-phenylpiperazine derivatives on hypothermia. In addition to blocking D_2 receptors, haloperidol at high doses also blocks serotonin 5-HT_2 receptors (K_d: D_2=0.4 nM; 5-HT_{2A}=46 nM) (Matsui-Sakata et al., 2005). Schotte et al. (1993) showed that haloperidol occupies D_2 and α_1 receptors at low doses (ED_{50}=0.13 and 0.42 mg/kg, respectively) and 5-HT_2 receptors at a higher dose (ED_{50}=2.6 mg/kg). Furthermore, administration of high-dose haloperidol (2.5–10 mg/kg, i.p.) prompted a reduction in mouse core temperature that was reversed by (±)DOI (0.5 mg/kg, i.p.) (Yamada et al., 1995). Thus, it is plausible that this synergistic effect between these *N*-phenylpiperazine derivatives and haloperidol is modulated by the serotonergic system.

This assumption was initially investigated by using the 5-$HT_{2A/C}$ agonist (±)DOI. Pretreatment with (±)DOI reduced the test compounds' hypothermic effects at 15 and 30 mg/kg, i.p. (Figs. 1C and 2C). Consequently, it is likely that LASSBio-579 and LASSBio-581 induced hypothermia, at least in part, through the serotonin 5-HT_2 receptor. However, (±)DOI enhanced the hypothermic effects of 60 mg/kg LASSBio-579 and LASSBio-581 (Figs. 1C and 2C), suggesting that a second mechanism may underlie the reduction in core temperature induced by these heterocyclic *N*-phenylpiperazine derivatives.

This somewhat puzzling result might be related to the functional interaction between serotonin receptors. 5-HT_2 and 5-HT_{1A} receptors are expressed in the same neuronal populations (Araneda and Andrade, 1991; Amargos-Bosch et al., 2004). Several data indicate that stimulation of 5-HT_{2A} receptors could mitigate 5-HT_{1A} receptor stimulation (Backus et al., 1990; Krebs-Thomson and Geyer, 1998; Stahl, 2000; Ichikawa et al., 2001; Meltzer et al., 2003). Thus, if serotonin 5-HT_{2A} receptors are blocked, rather than stimulated, the normal inhibitory influence on serotonin 5-HT_{1A} receptor stimulation is lost. This action may indirectly augment the effect of stimulating serotonin 5-HT_{1A} receptors because 5-HT_{1A} stimulation is no longer attenuated by 5-HT_{2A} stimulation (Stahl, 2000). Consequently, a substance able to stimulate 5-HT_{1A} receptors at low doses, and able to block 5-HT_2 receptors at high doses, could have a dual effect on responses mediated by 5-HT_{1A} receptors linked to 5-HT_2 receptor activation.

Hypothermia occurring as a consequence of serotonin 5-HT_{1A} receptor activation is the best characterized effect of serotonergic agents upon body temperature (Goodwin et al., 1985; Forster et al., 1995). Agonists at this receptor subtype, such as 8-OH-DPAT [8-hydroxy-2-(di-*n*-propylamino)tetralin], show a dose-dependent hypothermic response that has been used as an *in vivo* marker of serotonin 5-HT_{1A} receptor activation (Millan et al., 1993; Salmi and Ahlenius, 1998), an effect attenuated by coadministration of (±)DOI (Maswood and Uphouse, 1997; Kitamura et al., 2003).

These results led us to hypothesize that activation of serotonin 5-HT_{1A} receptors could mediate the hypothermic effects of LASSBio-579 and LASSBio-581. Confirming this hypothesis, WAY 100635 completely abolished the hypothermic effect of LASSBio-579 (Fig. 1D) and strongly diminished hypothermia induced by LASSBio-581 (Fig. 2D).

The increase in the hypothermic effects of 60 mg/kg LASSBio-581 and LASSBio-579 caused by (±)DOI coadministration possibly reflects an inhibitory action of these *N*-phenylpiperazine derivatives on 5-HT_2 receptor function. This assumption was corroborated by the observation that these compounds inhibited (±)DOI-induced head-twitches and ear-scratches (Tables 1 and 2), behaviors classically linked to 5-HT_{2A} receptor activation (Darmani et al., 1990a,b).

In addition to serotonin 5-HT_{2A} receptor antagonists, other agents, such as serotonin 5-HT_{1A} receptor agonists and α_1 receptor antagonists, can reduce the number of (±)DOI-induced head-twitches (Darmani et al., 1990a; Dursun and Handley, 1996). Thus, inhibition of the (±)DOI-induced head-twitch response by LASSBio-579 and LASSBio-581 may reflect serotonin 5-HT_2 receptor blockade concomitant with serotonin 5-HT_{1A} receptor activation. The effects of LASSBio-579 and LASSBio-581 on the number of ear-scratches confirms the involvement of 5-HT_2 receptors.

Altogether, the present results clearly indicate that LASSBio-579 and LASSBio-581 act on the serotonergic system. Furthermore, previous data published by our group (Menegatti et al., 2003; Neves et al., 2003) also point to the involvement of the dopaminergic system in the actions of LASSBio-579 and LASSBio-581. The literature suggests that such a pharmacological profile is promising when investigating new atypical antipsychotic drugs with favorable side-effects profiles.

Serotonin 5-HT_{2A} receptors differentially modulate the activity of dopaminergic neurons, depending on the brain region in question, and serotonin 5-HT_2 receptor antagonism

may provide an atypical profile for antipsychotic drugs that also presents weaker antidopaminergic action (Meltzer et al., 2003). For example, clozapine induced an increase in dopamine levels in the mouse prefrontal cortex through 5-HT_2 serotonin receptor blockade, with smaller increases in the striatum and nucleus accumbens, an effect considered to be critical for its ability to improve cognition and negative symptoms (Kapur and Remington, 1996; Meltzer et al., 2003). Evidence also exists that atypical antipsychotics acting at serotonin 5-HT_{2A} receptors may improve the mood state of schizophrenic patients (Stahl, 2000; Meltzer et al., 2003).

Recently, attention has been given to the serotonin 5-HT_{1A} receptor as a promising target for antipsychotic therapy. Rather than being selective agents, 5-HT_{1A} partial agonists with antidopaminergic action have elicited interest (Millan, 2000; Bruins Slot et al., 2005). The combination of D_2 antagonism and 5-HT_{1A} agonism induces neurochemical and behavioral changes that may be useful for ameliorating negative and cognitive symptoms with diminished extrapyramidal side effects and perhaps enhanced efficacy in refractory patients (Wadenberg, 1996; Ichikawa and Meltzer, 1998; Prinssen et al., 1999; Millan, 2000; Haleem et al., 2004; Assié et al., 2005).

In conclusion, LASSBio-579 and LASSBio-581 may represent potential lead atypical antipsychotic drugs. Serotonin receptor binding studies and investigation of the effects of these compounds in animal models of the negative symptoms of schizophrenia should further substantiate the usefulness of this molecular scaffold in the search for new antipsychotic drugs.

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