Brazilian Journal of Medical and Biological Research (2001) 34: 675-682
ISSN 0100-879X

Strain-dependent effects of diazepam and the 5-$HT_{2B/2C}$ receptor antagonist SB 206553 in spontaneously hypertensive and Lewis rats tested in the elevated plus-maze

R.N. Takahashi[1], O. Berton[2,3], P. Mormède[2] and F. Chaouloff[2]

[1]Departamento de Farmacologia, Universidade Federal de Santa Catarina, Florianópolis, SC, Brasil
[2]NeuroGénétique et Stress, INSERM U471-INRA, Bordeaux, France
[3]UCB Pharma, Preclinical CNS Research, Braine-L'Alleud, Belgium

Abstract

The 5-$HT_{2B/2C}$ receptor antagonist SB 206553 exerts anxiolytic effects in rat models of anxiety. However, these effects have been reported for standard rat strains, thus raising the issue of SB 206553 effects in rat strains displaying different levels of anxiety. Herein, the effects of SB 206553 in a 5-min elevated plus-maze test of anxiety were compared to those of the reference anxiolytic, diazepam, in two rat strains respectively displaying high (Lewis rats) and low (spontaneously hypertensive rats, SHR) anxiety. Diazepam (0.37, 0.75, or 1.5 mg/kg; 30 min before testing) increased in a dose-dependent manner the behavioral measures in SHR, but not in Lewis rats. On the other hand, SB 206553 (1.25, 2.5, or 5 mg/kg; 30 min before testing) failed to alter the anxiety parameters in both strains, whereas it increased closed arm entries in Lewis rats, suggesting that it elicited hyperactivity in the latter strain. Accordingly, the hypolocomotor effect of the nonselective 5-$HT_{2B/2C}$ receptor agonist m-chlorophenylpiperazine (1.5 mg/kg *ip* 20 min before a 15-min exposure to an activity cage) was prevented by the 1.25 and 2.5 mg/kg doses of SB 206553 in Lewis rats and SHR, respectively. Compared with SHR, Lewis rats may display a lower response to benzodiazepine-mediated effects and a more efficient control of locomotor activity by 5-$HT_{2B/2C}$ receptors.

Correspondence
R.N. Takahashi
Departamento de Farmacologia, UFSC
R. Ferreira Lima, 82
88015-420 Florianópolis, SC
Brasil
Fax: +55-48-222-4164
E-mail: takahashi@farmaco.ufsc.br

Presented at the XIII Annual Meeting of the Federação de Sociedades de Biologia Experimental, Caxambu, MG, Brazil, August 26-29, 1998.

Received September 15, 2000
Accepted February 5, 2001

Key words
- Anxiety
- Diazepam
- Elevated plus-maze
- Lewis rats
- Spontaneously hypertensive rats
- Locomotor activity
- m-Chlorophenylpiperazine
- SB 206553

Introduction

Early reports that the acute peripheral administration of the nonselective 5-$HT_{2B/2C}$ receptor agonist m-chlorophenylpiperazine (mCPP) promotes or exacerbates anxiety-related behaviors in laboratory animals and humans, respectively, have led to the suggestion that these receptors - at least those located in the hippocampus (1) - play a role in the modulation of anxiety (for a review, see 2). The initial use of nonselective antagonists for 5-$HT_{2B/2C}$ receptors (3,4), followed by analysis of the behavioral effects of SB

200646A, a selective, albeit weak, antagonist of these receptors (5), has indicated that blockade of 5-$HT_{2B/2C}$ receptors may actually be endowed with anxiolytic properties (6). Such an intrinsic property of 5-$HT_{2B/2C}$ receptor antagonists has raised interest with regard to the therapy of anxiety disorders. Accordingly, more potent antagonists have been synthesized, among them SB 206553, which displays both selectivity and high affinity for 5-$HT_{2B/2C}$ receptors (7). Studies comparing the anxiolytic effects of SB 206553 with those of standard anxiolytics, e.g., benzodiazepines, have concluded that SB 206553 exerts anxiolytic effects in unconditioned (rat social interaction test, rat elevated plus-maze test) and conditioned (rat Geller-Seifter test, rat Vogel test, marmoset conflict test) tests (7,8).

In a study aimed at comparing several components of central serotonergic systems in rat strains respectively displaying high (Lewis rat) and low (spontaneously hypertensive rat, SHR) anxiety in the elevated plus-maze, it was found that the 5-$HT_{2B/2C}$ receptor-mediated hypolocomotor effect of mCPP (9) was of similar potency in these two strains (10). The hypothesis that differences in anxiety between SHR and Lewis rats are mediated by 5-$HT_{2B/2C}$ receptors (see above) could not be examined because the high anxiety levels of Lewis rats prevented any inter-strain analysis of the anxiogenic effects of mCPP in the elevated plus-maze (10). However, since the completion of that study, antagonists for 5-$HT_{2B/2C}$ receptors such as SB 206553 have become available. Thus, in the present study we examined the hypothesis that the control exerted by 5-$HT_{2B/2C}$ receptors on anxiety-related behaviors is of greater impact in Lewis rats than in SHR. To this end, we assessed the behavioral effects of SB 206553 administration to Lewis rats and SHR exposed to an elevated plus-maze test, and compared these effects to those elicited by the standard anxiolytic, diazepam (11).

Material and Methods

Subjects

Male SHR and Lewis rats (IFFA CREDO, Les Oncins, France), 6-8 weeks old on arrival at our laboratory, were housed 3-4 per cage with food and water *ad libitum* under constant temperature (22 ± 2ºC) and on a 12-h light/12-h dark cycle (lights on at 7:00 h). All rats, which were used only once, were left undisturbed for at least 2 weeks before the beginning of the experiments. The protocol followed the rules established by the French legislation on animal welfare (publication J.O. 87-848).

Procedure

All experiments were performed between 13:00 h and 16:00 h. In a first series of experiments, rats were pretreated *ip* (2 ml/kg) either with vehicle (a few drops of Tween 80 in water) or with diazepam (0.37, 0.75, or 1.5 mg/kg in vehicle; Hoffmann-La Roche, Basel, Switzerland), and returned to their home cages for 30 min before being tested for 5 min in an elevated plus-maze. In the second series of experiments, rats were pretreated *ip* (2 ml/kg) either with the aforementioned vehicle or with SB 206553 (1.25, 2.5, or 5 mg/kg in vehicle; SmithKline Beecham Pharmaceuticals, Harlow, England) and returned to their home cages for 30 min before being tested for 5 min in the elevated plus-maze. At the end of the latter test, rats were injected *ip* (1 ml/kg) with either 0.9% NaCl or mCPP (1.5 mg/kg; RBI Bioblock, Illkirch, France) and returned to their home cages for another 20 min before being placed in activity cages for 15 min. The goal of this additional experiment was to test the efficiency of SB 206553 as a 5-$HT_{2B/2C}$ receptor antagonist. In all cases (first and second series of experiments), the animals were randomly assigned an order of testing.

Material

As described previously (12), the elevated plus-maze was made of Perspex, with four elevated arms (66 cm from the floor) 44-cm long and 10-cm wide. The arms were arranged in a cross-like disposition, with two opposite arms being enclosed by 50-cm high walls, and the two other arms being open, having at their intersection a central square platform (10 x 10 cm) which gave access to any of the four arms. All floor surfaces were black, and illumination was set at 70 lux. Each rat was placed on the central platform facing an open arm, and the number of entries to, and time spent on, each arm was assessed by video-recording. The activity cages, similar to the home cages (to reduce the impact of novelty), were placed inside a rack (8-lux illumination) equipped with two sets of infrared lights and photocell detectors connected to a computer which recorded the numbers of horizontal and vertical light beam interruptions (12).

Statistical analysis

All results are reported as means ± SEM. Because these data did not follow normal distribution, in each strain, behaviors in the open arms were compared by means of a Kruskal-Wallis analysis followed, if significant, by Dunn tests. The numbers of closed and total arm entries in the elevated plus-maze, and locomotor activity scores in the activity cages, all following normality, were compared by one-way ANOVA followed, if significant, by Tukey's multiple comparison test.

Results

Behavioral effects of diazepam

As shown in Figure 1, diazepam treatment (30 min before testing) did not affect the number of open arm entries in Lewis rats, although positive trends could be noted at the highest dose. On the other hand, total [$F(3,34) = 3.33$, $P = 0.031$], but not closed, arm entries were affected by diazepam treatment in this same strain, with the highest dose exerting a significant stimulatory effect (Figure 1). With regard to SHR, this treatment affected both percent number of open arm entries [$H = 12.93$, $P = 0.0048$] and percent time spent in these arms [$H = 14.41$, $P = 0.0024$]; actually, the 0.75 and 1.5 mg/kg doses of diazepam significantly increased these two variables (Figure 1). In addition, the 0.75 mg/kg dose of diazepam was found to decrease the number of closed arm entries [$F(3,34) = 2.89$, $P = 0.0498$ for the overall influence of diazepam], whereas total arm entries remained unaffected by diazepam treatment. Last, inter-strain comparisons between vehicle-treated rats revealed a higher percent time spent in the open arms by SHR compared to Lewis rats (Figure 1).

Behavioral effects of SB 206553

As shown in Figure 2, SB 206553 treatment (30 min before testing) did not affect the number of open arm entries of Lewis rats and SHR; conversely, it exerted a significant stimulatory effect on the number of closed arms explored by Lewis rats [$F(3,31) = 5.06$, $P = 0.0057$] and the total number of visits by Lewis rats [$F(3,31) = 3.43$, $P = 0.029$] and SHR [$F(3,31) = 5.81$, $P = 0.0028$]. *Post hoc* tests indicated that the first and third doses of SB 206553 on the one hand, and the second and third doses of SB 206553 on the other hand, increased closed arm exploration by Lewis rats and total arm exploration by Lewis rats and SHR, respectively (Figure 2). In that series of experiments, open arm entries and total arm exploration were found to be of greater amplitude in SHR compared to Lewis rats (Figure 2).

Figure 3 shows the effects of mCPP (1.5 mg/kg 20 min before testing) on vehicle- and SB 206553-pretreated (35 min before mCPP)

rats placed in activity cages for 15 min. These animals were previously tested to evaluate the effects of vehicle or SB 206553 in the plus-maze test. In each strain, the experimental groups were found to differ [$F(4,36) = 12.95$, $P<0.0001$ and $F(4,36) = 7.35$, $P = 0.0002$ for Lewis rats and SHR, respectively], with mCPP decreasing locomotor activity in vehicle-pretreated rats (Figure 3). Actually, this inhibitory effect of mCPP, which was found to be strain independent (62 ± 9 and 44 ± 9% reductions in the activities of SHR and Lewis rats, respectively; P = nonsignificant), was prevented by the 1.25 and 2.5 mg/kg doses of SB 206553 in Lewis rats and SHR, respectively (Figure 3). Moreover, the highest dose of SB 206553 was found to stimulate locomotor activity in mCPP-treated Lewis rats, but not in their SHR counterparts, when compared with rats pretreated with vehicle first and then with saline (Figure 3).

Discussion

The main goal of the present study was to explore the acute consequences of 5-$HT_{2B/2C}$ receptor blockade in Lewis rats and SHR exposed to an elevated plus-maze test of anxiety. For comparison, the behavioral effects of diazepam, a standard anxiolytic, were also investigated. It was observed that diaz-

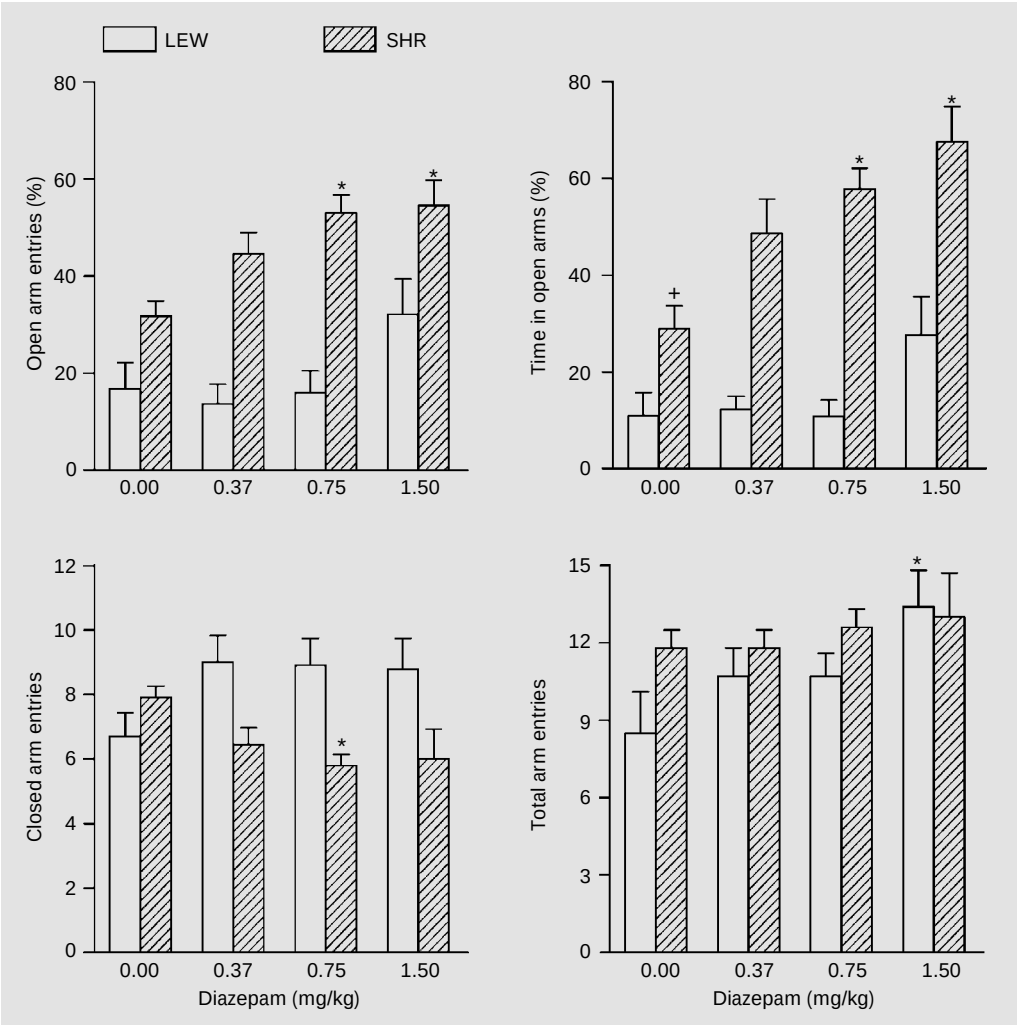

Figure 1 - Effects of diazepam administered *ip* (30 min before testing) in Lewis (LEW) and spontaneously hypertensive rats (SHR) tested for 5 min in the elevated plus-maze. Results are reported as the mean ± SEM for 9-10 rats. $^{+}P<0.05$ for the inter-strain difference between vehicle-treated rats (Tukey test). *P<0.05, at least for the effects of diazepam against vehicle (Dunn or Tukey test).

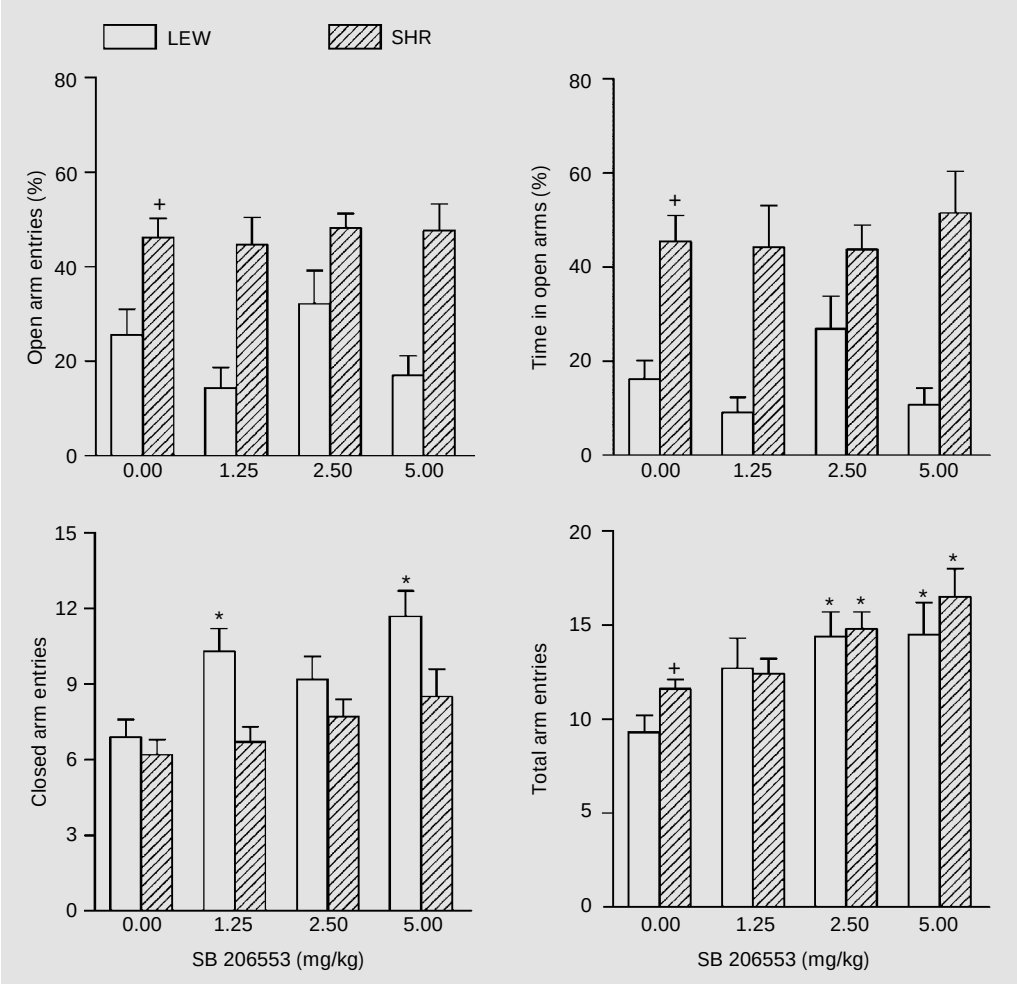

Figure 2 - Effects of SB 206553 administered *ip* (30 min before testing) in Lewis (LEW) and spontaneously hypertensive rats (SHR) tested for 5 min in the elevated plus-maze. Results are reported as the mean ± SEM for 6-10 rats. $^{+}$P<0.05 for the inter-strain difference between vehicle-treated rats (Dunn or Tukey test). *P<0.05, at least for the effects of SB 206553 against vehicle (Tukey test).

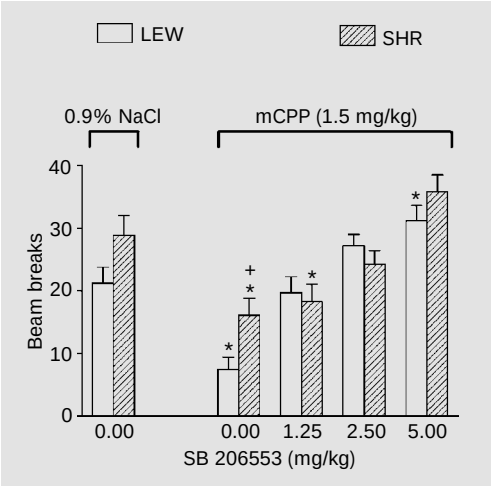

Figure 3 - Effects of m-chlorophenylpiperazine (mCPP) and saline administered *ip* on vehicle- and SB 206553-pretreated Lewis (LEW) and spontaneously hypertensive rats (SHR) 20 min before testing in activity cages for 15 min. These animals were immediately injected with saline or mCPP after completion of the plus-maze test (same rats as in Figure 2). Results are reported as the mean ± SEM for 6-10 rats. $^{+}$P<0.05 for the inter-strain difference between vehicle-pretreated (mCPP-injected) rats (Tukey test). *P<0.05, at least for the difference from vehicle-pretreated (saline-injected) rats (Tukey test).

epam-treated Lewis rats, as opposed to diazepam-treated SHR, did not display anxiolysis, as assessed by the measurement of the percent number of entries into, and percent time spent on, open arms (11). It is intriguing to note that genetically anxious rats did not respond to diazepam, whereas SHR, which display low anxiety (10,12), an observation again confirmed herein, proved to be sensitive to the anxiolytic. We previously reported that 2 mg/kg of diazepam (*ip*, 30 min before testing) triggered anxiolysis in both Lewis rats and SHR, with the effect being stronger in Lewis rats (12). On the basis of the present observation that the 1.5 mg/kg dose of diazepam tended to increase the number of open arm entries by Lewis rats, it may be concluded that Lewis rats are hyposensitive, rather than resistant, to the anxiolytic effects of diazepam (at least when compared with SHR). In addition, it is noteworthy that i) diazepam-treated SHR displayed lower locomotion in the closed arms, thus indicating that the diazepam-elicited increase in open arm exploration was not due to some motor stimulant effect, and that ii) diazepam-pretreated Lewis rats tended to display increased closed arm exploration, in agreement with our previous observation that a 2-mg/kg dose of diazepam increased the number of closed arm entries by Lewis rats in a strain-dependent manner (12). The nature of the mechanisms underlying the hyposensitivity of Lewis rats compared with SHR, with regard to the anxiolytic effects of diazepam remains to be determined. A first possibility is that this hyposensitivity may be due to altered pharmacokinetics of diazepam; it should be noted, however, that the 1.5 mg/kg dose of diazepam exerted significant stimulatory effects on the number of total arm entries (a mixed index of anxiety and activity; 12-14) in Lewis rats, thereby indicating that a significant amount of diazepam reached its central targets. Another mechanism could be a down-regulation of benzodiazepine-binding sites and/or a desensitization of benzodiazepine-mediated functions compared with SHR. Unfortunately, the only information available concerns benzodiazepine-binding sites in the hypothalamus of Lewis rats compared with Fischer 344 rats: actually, [^{3}H]-flunitrazepam binding was found to be higher in the former strain (15). However, whether such a difference extends to limbic regions, especially those involved in the anxiolytic effects of benzodiazepines in the elevated plus-maze (e.g., basolateral amygdala, septum; 16,17) when compared to SHR, remains to be explored.

In the present study, pretreatment with the 5-$HT_{2B/2C}$ receptor antagonist proved to be ineffective with respect to the plus-maze behavior of Lewis rats and SHR, indicating a lack of anxiolytic effect of SB 206553. Whether such inefficiency is due to our experimental setting is unlikely since diazepam-elicited anxiolysis could be observed in SHR; however, because baseline anxiety levels in SHR did differ between the two series of experiments, the possibility that SB 206553-elicited anxiolysis, if any, depends on baseline levels of anxiety cannot be ignored. To our knowledge, only one study has examined the consequences of SB 206553 treatment on elevated plus-maze behaviors. Indeed, it was observed that a 3-mg/kg dose, but not 1- or 10-mg/kg doses, given *ip* 30 min before testing, increased the number of open arm and total arm entries (8). It is likely that strain differences between the latter study (Sprague-Dawley) and the present one partially/fully account for these differences. Moreover, the lack of an anxiolytic effect of SB 206553 cannot be accounted for by the drug preparation and/or the doses used; indeed, SB 206553 increased closed arm entries in Lewis rats, and total arm entries in Lewis rats and SHR. In keeping with past evidence indicating that closed arm entries are an index of locomotor activity (12-14), elevated plus-maze experiments thus suggested that the Lewis rat strain may be sensitive to the hyperlocomotor effect of SB

206553. This observation confirms the notion that the effects of SB 206553 on anxiety and locomotor activity may be independent (7). In addition, the recent report that SB 206553 (1-4 mg/kg administered *ip* 30 min before animal placement in activity cages) did not affect the locomotor activity of Sprague-Dawley rats (18) reinforces the above hypothesis that the Lewis strain is sensitive to SB 206553-elicited hyperactivity. Confirming the strain-dependent effect of SB 206553 on closed arm entries, it was observed that SB 206553 pretreatment was more effective in Lewis rats than in SHR in counteracting mCPP-elicited hypolocomotion, a behavior thought to be mediated by 5-$HT_{2B/2C}$ receptors (9). Thus, a 1.25-mg/kg dose of SB 206553 was needed in Lewis rats to prevent mCPP-induced hypolocomotion while at least a double dose was necessary to be effective in SHR. As indicated in Results, such a strain-dependent difference could not be accounted for by the strain differences in the effects of mCPP since mCPP-elicited hypolocomotion, expressed as percent decreases compared to saline-treated rats, did not differ between SHR and Lewis rats (thus confirming a previous report; 10). Mechanisms other than 5-$HT_{2B/2C}$ receptors which could explain this strain-dependent difference in activity are unknown. However, recent microdialysis and electrophysiological studies have indicated that the blockade of 5-$HT_{2B/2C}$ receptors by systemically administered SB 206553 increases the basal firing rate of ventral tegmental area and nigral dopamine neurons and stimulates dopamine release from accumbal and striatal nerve terminals (19-21). Our study thus opens the interesting possibility that the tonic inhibitory control of mesolimbic and mesostriatal dopaminergic systems by 5-$HT_{2B/2C}$ receptors is more intense in Lewis rats than in SHR.

Acknowledgments

We thank Dr. André Ramos for helpful comments on the manuscript.

References

1. Whitton P & Curzon G (1990). Anxiogenic-like effect of infusing 1-(3-chlorophenyl) piperazine (mCPP) into the hippocampus. *Psychopharmacology*, 100: 138-140.
2. Kennett GA (1993). 5-HT_{1C} receptors and their therapeutic relevance. *Current Opinion in Investigation Drugs*, 2: 317-362.
3. Kennett GA (1992). 5-HT_{1C} receptor antagonists have anxiolytic-like actions in the rat social interaction model. *Psychopharmacology*, 107: 379-384.
4. Kennett GA, Pittaway K & Blackburn TP (1994). Evidence that 5-HT_{2C} receptor antagonists are anxiolytic in the rat Geller-Seifter model of anxiety. *Psychopharmacology*, 114: 90-96.
5. Forbes IT, Kennett GA, Gadre A, Ham P, Hayward CJ, Martin RT, Thompson M, Wood MD, Baxter GS, Glen A, Murphy OE, Stewart B & Blackburn TP (1993). N-(1-Methyl-5-indolyl)-N'-(3-pyridyl)urea hydrochloride: the first selective 5-HT_{1C} receptor antagonist. *Journal of Medicinal Chemistry*, 36: 1104-1107.
6. Kennett GA, Bailey F, Piper DC & Blackburn TP (1995). Effect of SB 200646A, a 5-HT_{2C}/5-HT_{2B} receptor antagonist, in two conflict models of anxiety. *Psychopharmacology*, 118: 178-182.
7. Kennett GA, Wood MD, Bright F, Cilia J, Piper DC, Gager T, Thomas D, Baxter GS, Forbes IT, Ham P & Blackburn TP (1996). *In vitro* and *in vivo* profile of SB 206553, a potent 5-HT_{2C}/5-HT_{2B} receptor antagonist with anxiolytic-like properties. *British Journal of Pharmacology*, 117: 427-434.
8. Griebel G, Perrault G & Sanger DJ (1997). A comparative study of the effects of selective and non-selective 5-HT_2 receptor subtype antagonists in rat and mouse models of anxiety. *Neuropharmacology*, 36: 793-802.
9. Kennett GA & Curzon G (1988). Evidence that mCPP may have behavioral effects mediated by 5-HT_{1C} receptors. *British Journal of Pharmacology*, 94: 137-147.
10. Kulikov A, Aguerre S, Berton O, Ramos A, Mormède P & Chaouloff F (1997). Central serotonergic systems in the spontaneously hypertensive and Lewis rat strains that differ in the elevated plus-maze test of anxiety. *Journal of Pharmacology and Experimental Therapeutics*, 281: 775-784.
11. Pellow S, Chopin P, File SE & Briley M (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, 14: 149-167.
12. Ramos A, Berton O, Mormède P & Chaouloff F (1997). A multiple-test study of anxiety-related behaviours in six inbred rat strains. *Behavioural Brain Research*, 85: 57-69.
13. File SE (1991). The biological basis of anxiety. In: Meltzer HY & Nerozzi D (Editors), *Current Practices and Future Developments in the Pharmacotherapy of Mental Disorders*. Elsevier, Amsterdam, The Netherlands, 159-165.

14. Cruz APM, Frei F & Graeff F (1994). Ethopharmacological analysis of rat behavior on the elevated plus-maze. *Pharmacology, Biochemistry and Behavior*, 49: 171-176.
15. Smith CC, Hauser E, Renaud NK, Leff A, Aksentijevich S, Chrousos GP, Wilder RL, Gold PW & Sternberg EM (1992). Increased hypothalamic [^{3}H]-flunitrazepam binding in hypothalamic-pituitary-adrenal axis hyporesponsive Lewis rats. *Brain Research*, 569: 295-299.
16. Pesold C & Treit D (1994). The septum and amygdala differentially mediate the anxiolytic effects of benzodiazepines. *Brain Research*, 638: 295-301.
17. Pesold C & Treit D (1995). The central and basolateral amygdala differentially mediate the anxiolytic effects of benzodiazepines. *Brain Research*, 671: 213-221.
18. McCreary AC & Cunningham KA (1999). Effects of the 5-HT$_{2C/2B}$ antagonist SB 206553 on hyperactivity induced by cocaine. *Neuropsychopharmacology*, 20: 556-564.
19. Lejeune F, Gobert A, Rivet JM & Millan MJ (1997). Serotonin (5-HT)$_{2C}$ receptors inhibit the activity of mesocortical and mesolimbic dopaminergic pathways: a combined dialysis and electrophysiological analysis. *Society for Neuroscience Abstracts*, 23: 975.
20. De Deurwaerdère P & Spampinato U (1999). Role of serotonin$_{2A}$ and serotonin$_{2B/2C}$ receptor subtypes in the control of accumbal and striatal dopamine release elicited *in vivo* by dorsal raphe nucleus electrical stimulation. *Journal of Neurochemistry*, 73: 1033-1042.
21. Di Giovanni G, De Deurwaerdère P, Di Mascio M, Di Matteo V, Esposito E & Spampinato U (1999). Selective blockade of serotonin-2C/2B receptors enhances mesolimbic and mesostriatal dopaminergic function: a combined *in vivo* electrophysiological and microdialysis study. *Neuroscience*, 91: 587-597.