BRAZILIAN JOURNAL
OF MEDICAL AND BIOLOGICAL RESEARCH
www.bjournal.com.br

ISSN 0100-879X
Volume 43 (3) 182-267 March 2011

BIOMEDICAL SCIENCES
AND
CLINICAL INVESTIGATION

Braz J Med Biol Res, March 2011, Volume 44(3) 224-228

doi: 10.1590/S0100-879X2011007500016

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Brazilian Journal of Medical and Biological Research (2011) 44: 224-228
ISSN 0100-879X

Central 5-HT_{2A} receptors modulate the vagal bradycardia in response to activation of the von Bezold-Jarisch reflex in anesthetized rats

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Abstract

Activation of 5-hydroxytryptamine (5-HT) 5-HT_{1A}, 5-HT_{2C}, 5-HT_3, and 5-HT_7 receptors modulates the excitability of cardiac vagal motoneurones, but the precise role of 5-$HT_{2A/2B}$ receptors in these phenomena is unclear. We report here the effects of intracisternal (*ic*) administration of selective 5-$HT_{2A/2B}$ antagonists on the vagal bradycardia elicited by activation of the von Bezold-Jarisch reflex with phenylbiguanide. The experiments were performed on urethane-anesthetized male Wistar rats (250-270 g, N = 7-9 per group). The animals were placed in a stereotaxic frame and their atlanto-occipital membrane was exposed to allow *ic* injections. The rats received atenolol (1 mg/kg, *iv*) to block the sympathetic component of the reflex bradycardia; 20-min later, the cardiopulmonary reflex was induced with phenylbiguanide (15 µg/kg, *iv*) injected at 15-min intervals until 3 similar bradycardias were obtained. Ten minutes after the last pre-drug bradycardia, R-96544 (a 5-HT_{2A} antagonist; 0.1 µmol/kg), SB-204741 (a 5-HT_{2B} antagonist; 0.1 µmol/kg) or vehicle was injected *ic*. The subsequent *iv* injections of phenylbiguanide were administered 5, 20, 35, and 50 min after the *ic* injection. The selective 5-HT_{2A} receptor antagonism attenuated the vagal bradycardia and hypotension, with maximal effect at 35 min after the antagonist (pre-drug = -200 ± 11 bpm and -42 ± 3 mmHg; at 35 min = -84 ± 10 bpm and -33 ± 2 mmHg; $P < 0.05$). Neither the 5-HT_{2B} receptor antagonists nor the vehicle changed the reflex. These data suggest that central 5-HT_{2A} receptors modulate the central pathways of the parasympathetic component of the von Bezold-Jarisch reflex.

Key words: 5-HT_2 receptors; R-96544; Reflex bradycardia; SB-204741; Vagal motoneurons; von Bezold-Jarisch reflex

Introduction

A large body of evidence indicates the importance of serotonin (5-HT) as a neurotransmitter in the circuitry controlling blood pressure and heart rate, particularly in the brainstem (1). One obvious complication in the study of the physiological roles of 5-HT is that it acts on up to 14 subtypes of receptors, grouped into seven distinct classes (2). The main sources of 5-HT for the central control of the cardiovascular system are neurons located in the lower brainstem raphe nuclei (1). The serotonergic innervation of the medullary areas involved in autonomic cardiovascular control, such as the dorsal vagal nucleus (DVN) and the nucleus ambiguus (NA), has been well documented (1,3).

A useful approach to the investigation of the autonomic control of the heart consists of analyzing how the cardiovascular reflexes, such as the von Bezold-Jarisch reflex (BJR), are centrally modulated. The BJR, whose effects include bradycardia, hypotension and short-lived apnea, can be experimentally elicited by activating the peripheral vagal afferents with phenylbiguanide (PBG), a selective

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Received July 28, 2010. Accepted January 19, 2011. Available online February 4, 2011. Published March 7, 2011.

5-HT$_3$ receptor agonist (4,5) that does not cross the blood-brain barrier. The reflex bradycardia induced by the BJR is mostly of vagal origin, with a variable participation of the sympathetic component (1,6). If the experimental animal is pretreated with atenolol, a non-brain-penetrant β_1 receptor antagonist (7), the magnitude of the reflex bradycardia will indirectly estimate the excitability of the cardiac vagal motoneurons (4). Studies using similar protocols (atenolol-pretreated animals) have suggested that the excitation of central circuits involved in the control of the cardiac vagal motoneurons involves the activation of 5-HT$_{1A}$ (4), 5-HT$_{2C}$ (8), 5-HT$_3$ (9), and 5-HT$_7$ (10) receptors. This is an issue which has already been extensively reviewed (1,11,12).

Most of the existing studies have concentrated on the sympathetic activity of the subtypes of 5-HT$_2$ receptors (5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$) in the regulation of cardiovascular activity (1). It has been reported that non-selective centrally acting 5-HT$_2$ receptor antagonists reduce blood pressure in normotensive (13) and hypertensive (14) rats, with negligible effects on baseline heart rate. Although studies on the 5-HT$_{2A}$ receptors, particularly involving the nucleus ambiguus (main source of the cardiac vagal motoneurons) in reflex situations are scarce (1,12), there are a few studies on the role of subtypes of 5-HT$_2$ receptors in the excitability of the cardiac vagal motoneurons in phasic situations (i.e., cardiovascular reflexes, including the BJR). These studies have provided some evidence that the 5-HT$_{2B}$ receptors excite while the 5-HT$_{2C}$ receptors inhibit the neurons of the nucleus tractus solitarii (NTS) (1,8,12,15). In the present report, we describe the effects of central administration of the selective ligands R-96544 (a 5-HT$_{2A}$ receptor antagonist) (16) and SB-204741 (a 5-HT$_{2B}$ receptor antagonist) (17) on the reflex vagal bradycardia elicited by activation of cardiopulmonary efferents with *iv* PBG.

Material and Methods

All animal procedures adopted were in accordance with the Biomedical Research Guidelines for Care and Use of Laboratory Animals, as stated by the Federation of the Brazilian Societies of Experimental Biology (FESBE). The experimental protocol was approved by the Animal Use Committee at Escola Superior de Ciências da Santa Casa de Misericórdia (EMESCAM; 021/2007-CEUA/EMESCAM, Vitória, ES, Brazil).

Animals

The experiments were performed on anesthetized, spontaneously breathing, male Wistar rats weighing 250-270 g obtained from the breeding stock of the Universidade Federal do Espírito Santo. Anesthesia was induced with halothane and maintained with urethane (1.2 g/kg, *iv*) and supplementary doses of urethane were administered as required. The femoral artery was cannulated for the measurement of blood pressure with a pressure transducer (Viggo-Spectramed, P23XL, USA) and the heart rate was derived electronically from the blood pressure signal using a rate meter (Biotach, Gould 13-64616-66, USA). The left femoral vein was cannulated for drug administration. Rectal temperature was maintained between 37 and 37.5°C with a thermostatically controlled heating blanket (Harvard, USA). The animals were placed in a stereotaxic apparatus and the atlanto-occipital muscles were carefully removed in order to expose the atlanto-occipital membrane. A metal 27G needle connected to a PE-10 cannula was then introduced through the membrane so that drugs (or vehicle) could be administered intracisternally (*ic*).

Experimental protocol

After cannulation and surgery the animals were pretreated with atenolol (1 mg/kg, *iv*), a selective β_1-adrenoceptor antagonist, which does not bind to 5-HT receptors (18) and does not cross the blood-brain barrier (7). Therefore, changes in cardiac vagal preganglionic neuron activity could be indirectly inferred from changes in heart rate (4,9). Once blood pressure and heart rate had stabilized, a reflex (BJR) was induced with PBG (15 µg/kg, *iv*) and repeated every 15 min until three similar bradycardias were obtained. This dose of PBG was found to induce a submaximal response and did not show tachyphylaxis over the time intervals involved (4,9). Ten minutes after the third consistent bradycardia, 10 µL of the test drug (or vehicle) was administered *ic* with a Hamilton® syringe over a period of 20 s. The subsequent *iv* PBG injections were made at 5, 20, 35, and 50 min after the *ic* injection in order to monitor any changes in the amplitude of vagal bradycardia.

Statistical analysis

The control consisted of the mean of three reflex bradycardias elicited by PBG before the administration of the test drugs. The absolute changes in heart rate and blood pressure were subsequently measured. The background resting heart rate and mean blood pressure were measured 10 min before the administration of the test drug. All data are

Table 1. Baseline and post-drug test values of heart rate (HR) and mean arterial pressure (MAP) in the test groups.

Test groups	N	HR (bpm)		MAP (mmHg)	
		Baseline	Post-drug	Baseline	Post-drug
Saline (10 µL, *ic*)	8	355 ± 13	369 ± 17	98 ± 3	98 ± 3
R-96544 (0.1 µmol/kg, *ic*)	9	342 ± 10	350 ± 9	90 ± 4	87 ± 4
SB-204741 (0.1 µmol/kg, *ic*)	7	336 ± 17	381 ± 10*	88 ± 3	84 ± 2

*P < 0.05 compared to baseline HR (Student *t*-test).

reported as means ± SEM for 7-9 animals per group. The effects of *ic* drug injection on the baseline parameters were assessed by the paired Student *t*-test. Reflex changes in heart rate and in mean blood pressure for each experimental group were compared by two-way repeated measures ANOVA followed by Bonferroni's multiple comparison test. Differences were considered to be significant at $P < 0.05$.

Drugs

Drugs and sources were as follows: atenolol and urethane (Sigma, USA); phenylbiguanide (Aldrich, UK); R-96544 and SB-204741 (Tocris Cookson, USA). R-96544 chemically is (2*R*,4*R*)-5-[2-[2-[2-(3-methoxyphenyl)ethyl]phenoxy]ethyl]-1-methyl-3-pyrrolidinol hydrochloride. SB-204741 is N-(1-methyl-5-indolyl)-N'-(3-methyl-5-isothiazolyl)urea. The drugs were freshly prepared and injected using 0.9% saline as vehicle.

Results

Control heart rate and mean arterial pressure did not differ significantly among the three experimental groups (Table 1). Intracisternal injection of SB-204741, a potent and selective 5-HT_{2B} receptor antagonist, caused a mild and statistically significant increase (+13.3%) in the baseline heart rate without inducing any changes in baseline blood pressure. Neither the 5-HT_{2A} receptor antagonist R-96544 nor the *ic* administered vehicle significantly changed the baseline parameters (Table 1).

Pre-drug (control) episodes of reflex changes in heart rate and mean blood pressure elicited by *iv* phenylbiguanide (von Bezold-Jarisch reflex) did not differ significantly among the experimental groups (Figure 1).

The 5-HT_{2A} receptor antagonist attenuated the vagal bradycardia induced by phenylbiguanide (pre-drug = -200 ± 11 bpm; 5 min = -121 ± 21; 20 min = -85 ± 12; 35 min = -84 ± 10; 50 min = -107 ± 19 bpm; $P < 0.05$). Therefore, attenuation of the vagal bradycardia induced by R-96544 was maximal (-57.7%) at 20-35 min after *ic* injection of the antagonist, and persisted until the last measurement made at 50 min (Figure 1). On the other hand, the 5-HT_{2B} receptor antagonist or vehicle did not cause any obvious changes in the reflex parameters (e.g., pre-SB-204741 = -186 ± 11 bpm; 5 min = -185 ± 16; 20 min = -188 ± 19; 35 min = -208 ± 11; 50 min = -200 ± 19 bpm; Figure 1).

Discussion

The main finding of the present study is that central 5-HT_{2A} receptors (but probably not 5-HT_{2B} receptors)

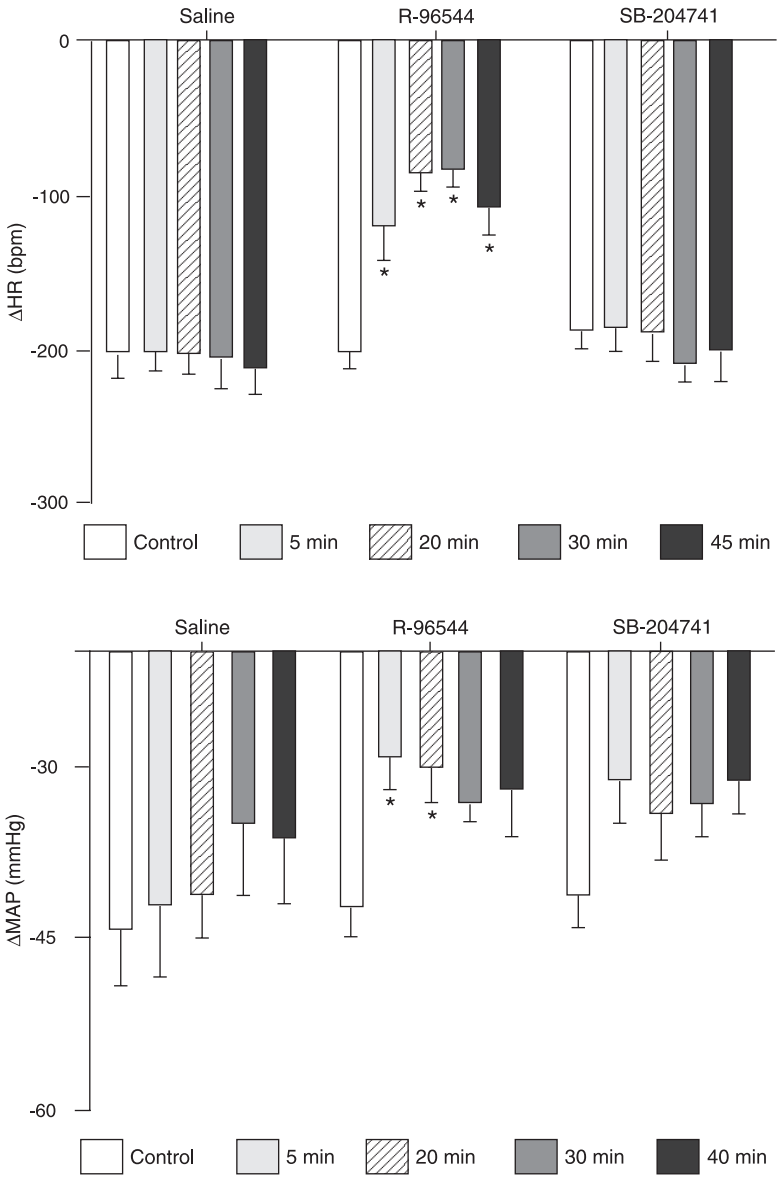

Figure 1. Reflex changes in heart rate (ΔHR) and in mean arterial pressure (ΔMAP) evoked by *ic* phenylbiguanide in atenolol-pretreated anesthetized rats. Control denotes the mean of the three cardiopulmonary reflex responses elicited by phenylbiguanide before the intracisternal (10 µL) administration of the test drug (or vehicle). *P < 0.05 compared to each respective control (*post hoc* Bonferroni multiple comparison test).

play a role in the modulation of the central pathways of the parasympathetic component of the von Bezold-Jarisch reflex in the rat. In this respect, 5-HT$_{2A}$ receptors behave similarly to 5-HT$_{1A}$, 5-HT$_3$ and 5-HT$_7$ receptors (1), albeit the mechanisms involved are unlikely to be the same. 5-HT$_2$ receptors are G-protein-coupled receptors, and their three subtypes are related in several aspects, including their signaling properties (2). On the other hand, 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors have a widespread distribution in the central nervous system, while the 5-HT$_{2B}$ receptors have a more restricted distribution (2,19).

It should be noted that the pioneering paper describing the participation of central 5-HT$_{1A}$ receptors in the excitability of cardiac vagal motoneurons (4) did not find any role of 5-HT$_2$ receptors in such phenomenon, since the non-selective 5-HT$_2$ receptor antagonist BW 501C67 did not modify the reflex bradycardia. At the time when the original study was carried out the distinction between the three subtypes of 5-HT$_2$ receptors was not completely understood, and selective ligands were not available (20). Although the binding profile of BW 501C67 and the lack of effect of BW 501C67 on the excitability of cardiac vagal motoneurons are still not fully understood, the drug is considered to be a 5-HT$_{2A/2C}$ receptor antagonist that does not cross the blood-brain barrier (21). This led us to speculate that the nonselective double blockage of 5-HT$_{2A}$/5-HT$_{2C}$ receptors by the drug might contribute to the process, a view that is corroborated by the information that both the 5-HT$_{2A}$ and the 5-HT$_{2B}$ receptors exert opposing effects on neurons of the NTS (12). We should mention a pioneering study by N'Diaye et al. (22), showing that DOI, a non-selective 5-HT$_2$ receptor agonist, when microinjected into the NTS of rats, enhanced NMDA receptor-mediated reflex bradycardia. These results agree with our hypothesis that 5-HT$_{2A}$ receptors facilitate the reflex control of heart rate, although the animals were not beta-blocked as in the present study.

A major concern is that the validity of our conclusions is highly dependent on the selectivity of the antagonists used, namely R-96544 and SB-204741. The R-96544 antagonist has a pA$_2$ = 10.4 for the 5-HT$_{2A}$ receptors, with low affinity for other subtypes of 5-HT receptors; furthermore, it practically does not bind to the dopamine or adrenergic receptors (16). Regarding SB-204741, the pK values for 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors are 5.3, 8.0, and 5.8, respectively (20,23). Therefore, the two ligands used in the present study are reasonably selective, making it difficult to attribute the lack of effect of SB-204741 on the reflex bradycardia to an insufficient dose. Moreover, SB-204741 caused a small, although statistically significant, increase in resting heart rate, suggesting that the 5-HT$_{2B}$ receptors could have a role in the tonic control of heart rate, even though they do not seem to act on reflex situations. However, there is no published study using these drugs by the *ic* route, and the doses used here were adapted from studies employing other routes (16,17,24). Therefore, a definitive answer concerning the role of 5-HT$_{2A}$ receptors (and the lack of a role of 5-HT$_{2B}$ receptors) in the modulation of vagal bradycardia will require that different selective antagonists be administered at different doses.

The two 5-HT$_2$ receptor antagonists used here changed the baseline blood pressure slightly, but these changes were not statistically significant. In fact, although the literature reports the difficulty in obtaining a reduction of blood pressure under normotensive conditions (1), a few studies have demonstrated hypotension in experimental animals after central antagonism of 5-HT$_2$ receptors (13,14). Since in the present study the rats were pretreated with atenolol, we suggest that the attenuation of the reflex reductions in mean arterial pressure observed after *ic* injection of R-96544 was mainly a consequence of the attenuation of the reflex bradycardia. Therefore, our main interest was focused on the cardiovagal component of the reflex, which can be inferred as an indirect measure of the excitability of the cardiac vagal motoneurons (4,9,10).

Cardiac vagal motoneurons are mainly located in the NA, but also in the DVN (1). These sites, together with the NTS, the first site of termination of afferent fibers originating from peripheral cardiopulmonary receptors, can be accessed by *ic* injections, as suggested by previous studies (1,9). Concerning our results with the 5-HT$_{2A}$ receptor antagonism, although our method is not able to pinpoint a specific anatomical site, the most probable targets are the NA, DVN and/or the NTS, possibly combined (12,15,24).

The role of 5-HT$_2$ receptors (particularly the 2A subtype) in the central modulation of reflex bradycardia seems to depend on the type of cardiovascular reflex. For instance, it has been recently reported that the antagonism of 5-HT$_{2A}$ receptors located in the NA increases (instead of decreasing) the vagal bradycardia observed in the diving response, which is a kind of trigeminocardiac reflex (25). The reasons for such a discrepancy can be attributed to the distinct anatomy of the pathways of these reflexes, as previously suggested for the 5-HT$_{1A}$ receptors (11).

We provide further evidence that central 5-HT$_{2A}$ receptors direct or indirectly modulate the excitability of cardiac vagal motoneurons. A next step will be to investigate how the several subtypes of 5-HT receptors compare regarding the excitability of cardiovagal inhibitory neurons. Distinct selective ligands will be necessary for this type of study, along with the use of microinjection into selective target areas, particularly the nucleus ambiguus.

Acknowledgments

The authors thank Mr. Mário A. Dantas (UFES) for technical assistance. Research partially supported by CNPq. S.M. Macedo was the recipient of a MSc CAPES fellowship.

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