Neuropeptides 45 (2011) 219–227

Contents lists available at ScienceDirect

Neuropeptides

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

Multiple opioid receptors mediate the hypotensive response induced by central 5-HT_3 receptor stimulation

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ARTICLE INFO

Article history:
Received 18 December 2010
Accepted 29 March 2011
Available online 22 April 2011

Keywords:
5-HT_3 receptors
Opioid receptors
Blood pressure

ABSTRACT

The aim of the present work was to investigate the role of brain μ, κ and δ opioid receptors in the central serotonergic mechanisms regulating blood pressure in rats. The data obtained show that: (1) pharmacological activation of central 5-HT_3 receptors yields a significant decrease in blood pressure; (2) the blockade of those receptors by a selective antagonist induces an acute hypertensive response; (3) the pharmacological blockade of central opioid receptors by three different opioid antagonists exhibiting variable degrees of selectivity to μ, κ and δ opioid receptors always suppressed the hypotensive response induced by central 5-HT_3 receptor stimulation; (4) the blockade of opioid receptors by the same opioid antagonists that impaired the hypotensive effect of central 5-HT_3 receptor stimulation failed to modify blood pressure in animals not submitted to pharmacological manipulations of central 5-HT_3 receptor function. It is shown that a 5-HT_3 receptor-dependent mechanism seems to be part of the brain serotonergic system that contributes to cardiovascular regulation since the hypertensive response observed after ondansetron administration indicates that central 5-HT_3 receptors exert a tonic inhibitory drive on blood pressure. Furthermore, the data obtained here clearly indicate that the hypotensive response observed after pharmacological stimulation of central 5-HT_3 receptors depends on the functional integrity of brain μ, κ and δ opioid receptors, suggesting that a functional interaction between serotonergic and opiatergic pathways in the brain is part of the complex, multifactorial system that regulates blood pressure in the central nervous system.

1. Introduction

Acting on multiple receptor subtypes operated by a complex and widely distributed circuitry, brain serotonin modulates several aspects of cardiovascular function, including blood pressure (Côté et al., 2004; Ramage, 2001; Ramage and Villalón, 2008). The effects produced by central serotonergic pathways on blood pressure depend on the subtype of receptors and the brain area studied (Ramage, 2001; Szabó et al., 1998). Serotonin-dependent modifications in blood pressure seem to be expressed by changes in autonomic nervous system function, such as sympathoexcitation or sympathoinhibition (Côté et al., 2004). Anticipatory and corrective adjustments in cardiovascular function to cope with visceral and behavioral challenges are under central serotonergic influence, and brain serotonin pathways seem to be crucial in allowing stress-induced hypertensive responses (Nalivaiko and Sgoifo, 2009).

We have previously demonstrated that central brain 5-HT_3 receptors exert tonic depressor effects on blood pressure in non-stressed rats, probably through a sympathoinhibitory-related mechanism (Ferreira et al., 2004). The effect of the 5-HT_3 agents on blood pressure control shown in that study may be partially explained by their action on the septal area, since when the same serotonergic agents were administered in the medial septum/vertical limb of the diagonal band complex (MS/vDB), 5-HT_3 receptors located in this area were also shown to exert a tonic sympathoinhibitory effect that seems to be mediated by an angiotensinergic-dependent mechanism (Urzedo-Rodrigues et al., 2011). Furthermore, it has also been shown that activation of central 5-HT_{2C} receptors induces hypertension in non-stressed rats and that the functional integrity of those receptors is essential for the rise in blood pressure that occurs in the course of restraint stress (Ferreira et al., 2005).

The central opioid system also participates in blood pressure regulation (Bodnar, 2009; Feuerstein and Sirén, 1988). However, a review of the literature reveals a rather controversial picture in which, depending on the opioid peptide, the receptor subtype, and the brain area studied different responses are obtained. Several experiments using methodological approaches based on the central administration of selective opioid agonists and antagonists have shown either hypotensive or hypertensive responses and, in some of them, no changes in this parameter (Vaccarino and Kastin,

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doi:10.1016/j.npep.2011.03.004

2001). These discrepancies may be due to differences in several aspects of the experimental protocols, in the pharmacological and pharmacokinetic properties of the compounds used, or the distinct sites of central injections. Nonetheless, consistent and expressive alterations in opioid function in spontaneously hypertensive rats seem to be very well documented, revealing that brain opioid peptides play an indisputable role in blood pressure regulation (De Wardener, 2001).

Functional interactions between central serotonergic and opiatergic pathways have been observed. Indeed, electrical stimulation of spinal neurons increases the synthesis and the release of opioid peptides, an effect that is blocked by selective 5-HT_{1A} receptor activation (Song et al., 2007) and ondansetron, a selective 5-HT_3 receptor antagonist, reduces opioid withdrawal behavior in both humans and rodents (Chu et al., 2009). In addition, serotonergic modulation of opiatergic function seems to be important in cardiovascular regulation since hypotension induced by selective inhibition of serotonin reuptake is blocked by opioid antagonists in spontaneously hypertensive rats (Goldstein et al., 1987). Furthermore, serotonin is necessary for the maintenance of normal levels of dynorphin mRNA in many areas of the brain (D'Addario et al., 2007).

Taking the above information into consideration, the aim of the present study was to investigate the possible participation of brain μ, κ and δ opioid receptor subtypes in the hypotensive response induced by the pharmacological stimulation of central 5-HT_3 receptors.

2. Methods

2.1. Animals

Adult male Wistar rats weighing 300 ± 20 g were used in the present study. They were kept under controlled light (lights on from 5 AM to 7 PM) and temperature (22 ± 2 °C) conditions, and had free access to tap water and laboratory chow (Nuvital Nutrientes Ltda., Curitiba, Brazil). All experimental sets were conducted in naïve rats. Groups of rats used in one experimental set were not reused in any other part of the study. The experimental protocols were performed according to the regulations established by the National Institutes of Health (USA).

2.2. Surgical procedures

Five days before the experimental sessions a guide cannula was implanted into the lateral ventricle (LV) under ketamine/xylazine (80/11.5 mg/kg i.p.) anesthesia. In brief, after positioning the rat in a stereotaxic apparatus (David Kopf Instruments, USA), a chronic 28-gauge guide cannula was implanted according to the following coordinates: anteroposterior = 1.2 mm posterior to the bregma; lateral = 1.5 mm; vertical = 4.0 mm below the skull. The guide cannula was fixed to the skull with metal screws and dental cement. After surgery, the animals were housed in individual cages. Two days before the experimental sessions, a catheter (PE50) filled with heparinized saline solution (1000 U/ml) was inserted into the left carotid artery under ketamine/xylazine anesthesia, and exteriorized at the nape of the animal's neck to permit blood pressure recording. The location of the guide cannula in the LV and the intracerebroventricular (ICV) injection site was confirmed at the end of the experiment with the use of Evans Blue dye injected through the cannula. The brains were removed, placed in formalin, and later frozen and cut into 40 μm sections. The slices were stained with cresyl violet and analyzed using light microscopy. Only data from the animals in which the tip of the cannula was restricted to the cerebroventricular space and the dye could not be seen in the brain tissue surrounding the ventricle were included in the study.

2.3. Drugs and microinjections

The following drugs were used: m-chlorophenylbiguanide hydrochloride (1-(3-chlorophenyl)biguanide; m-CPBG), a selective 5-HT3 agonist (Van Hooft and Vijverberg, 1997; Sepúlveda et al., 1991) was purchased from Tocris Cookson, Inc. Ballwin, MO. Ondansetron, a selective 5-HT_3 antagonist (Gaster and King, 1997; Costall and Naylor, 2004; Thompson and Lummis, 2006), was purchased from Sigma Chemical, Co., St. Louis, MO. Naloxone, an opioid antagonist preferentially binding to μ receptors, NOR-binaltorphimine (NOR-BNI), an opioid antagonist preferentially binding to κ receptors, and naltrindole, an opioid antagonist preferentially binding to δ receptors, were also acquired from Sigma Chemical, Co., St. Louis, MO. The doses of all drugs used in this study were compatible with the doses used by other research groups. All solutions were at neutral pH; no acid or basic solutions were injected. Central injections were given using a Hamilton microsyringe connected to a 30-gauge injector through polyethylene tubing. A total volume of 2 μl was slowly injected (60 s).

2.4. Blood pressure recording

Arterial pressure was continuously monitored through the carotid catheter connected to a blood pressure transducer (World Precision Instruments) whose signal was amplified and digitally recorded by an analog-to-digital interface (AqDados – application for data acquisition, Lynx Tecnologia Eletrônica Ltda, São Paulo, Brazil, version 7.0) and recorded (1 kHz) on a microcomputer for later analysis. Mean arterial pressure (MAP) was calculated from systolic and diastolic pressures data, while heart rate (HR) was determined from the pulsation of arterial pressure using the AcqKnowledge software program, version 3.5.7, developed by Biopac Systems, Inc., California, USA.

2.5. Experimental design

To study the effect of brain 5-HT_3 receptors on blood pressure, MAP was recorded in a group of rats receiving injections of the selective 5-HT_3 agonist m-CPBG at a dose of 160 nmol or saline solution (controls) into ICV. To verify whether the central serotonergic pathways would exert tonic control on blood pressure through their effect on 5-HT_3 receptors, MAP was recorded in a separate group of animals treated with ondansetron, a selective 5-HT_3 antagonist, at the dose of 80 nmol or saline solution (controls). Serotonergic drugs or isotonic saline solution (controls) were injected into ICV 30 min after baseline MAP was recorded.

Additionally, to investigate the possible participation of central opiatergic pathways in the hypotensive response induced by central 5-HT_3 receptor stimulation, separate groups of animals received ICV injections of m-CPBG at a dose of 160 nmol or saline solution (controls) 30 min after the pretreatment with ICV injections of distinct opioid antagonists: naloxone, an opioid antagonist preferentially binding to μ receptors (30 nmol), NOR-binaltorphimine, an opioid antagonist preferentially binding to κ receptors (10 nmol), and naltrindole, an opioid antagonist preferentially binding to δ receptors (1 nmol). The animals were allowed to move freely around their cages in all the experiments. Also, in all the experimental sets, MAP was recorded in the animals for 30 min prior to the administration of any drug to insure that baseline blood pressure was normal in each animal.

2.6. Statistical analysis

A computer software package (GraphPad, San Diego, CA) was used to perform two-way (treatment and time as factors) analysis of variance for repeated measures in each experimental set. The

post hoc Bonferroni test was used to compare each treatment. Differences between the groups were considered statistically significant when $p < 0.05$. The data are presented as means ± SEM. The MAP and HR values at the end of the stabilization period (time zero) were used as references to calculate the delta values that are presented throughout the experiments.

3. Results

Fig. 1 summarizes the effects on blood pressure and heart rate obtained after the injections of the serotonin 5-HT_3 receptor agonists and antagonists into the lateral ventricle. Panel A shows that in animals receiving lateral ventricle injections of saline + m-CPBG (160 nmol) a significant decrease in blood pressure was observed, as compared to controls (saline + saline). This hypotensive response is already evident 5 min after m-CPBG injection and lasts for the entire duration of the experiment. In animals receiving ondansetron (80 nmol) + saline into the lateral ventricle an acute hypertensive response is observed 5 min after the administration of the ondansetron and lasts for 2 h. Pretreatment with ondansetron was also found to impair the hypotensive response induced by m-CPBG injections into the lateral ventricle. No significant differences in HR were observed in any of the above mentioned groups, as shown in Panel B. Analysis of variance for MAP indicated a significant treatment and time effect and significant treatment × time interaction [$F_{(3,323)} = 80.64$, $p < 0.0001$; $F_{(17,323)} = 1720.41$, $p < 0.0001$; $F_{(19,323)} = 4.57$, $p < 0.0001$, respectively]. Analysis of variance for HR indicated a significant time effect, no significant treatment effect, and significant treatment × time interaction [$F_{(17,323)} = 155.13$, $p < 0.0001$; $F_{(3,323)} = 1.16$, $p = 0.3520$; $F_{(19,323)} = 10.48$, $p < 0.0001$, respectively].

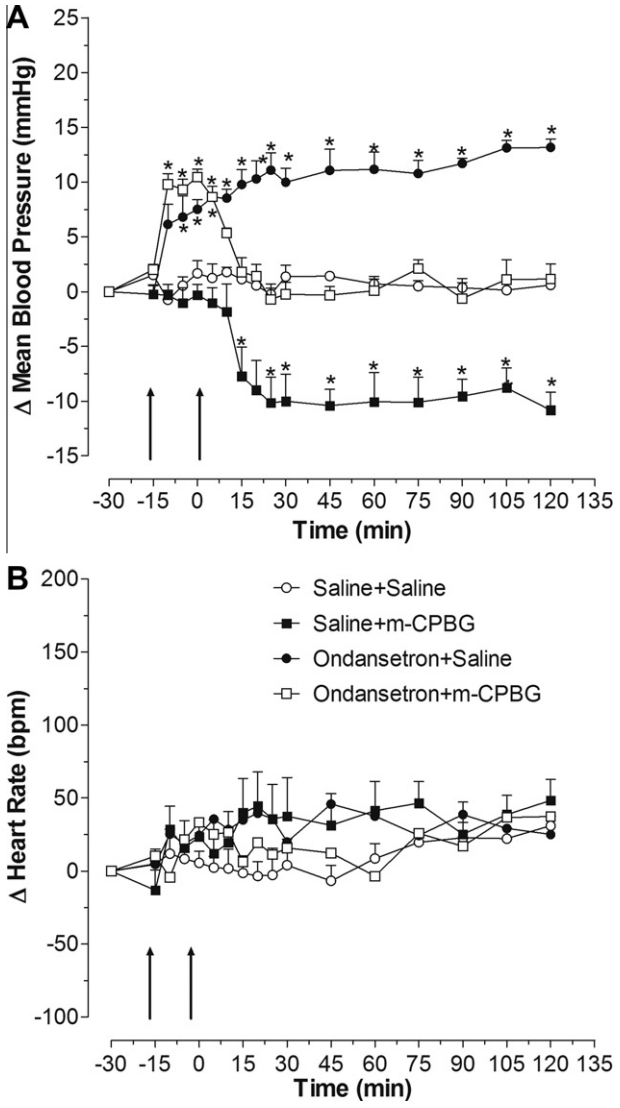

Fig. 1. Changes (Δ) in mean arterial pressure (MAP – Panel A) and heart rate (HR – Panel B) in animals receiving lateral ventricle injections of distinct 5-HT_3 receptor agonists and antagonists or saline (controls). Data are presented as means ± SEM. The following groups are presented: saline + m-CPBG ($n = 5$; basal MAP = 112.7 ± 4.5; basal HR = 330.3 ± 14.4); ondansetron + saline ($n = 6$; basal MAP = 117.1 ± 1.1; basal HR = 308.9 ± 19.5); ondansetron + m-CPBG ($n = 6$; basal MAP = 114.2 ± 1.7; basal HR = 312.7 ± 20.6); saline + saline ($n = 6$; basal MAP = 109.2 ± 3.0; basal HR = 317.0 ± 9.3). *Statistically significant differences (two-way ANOVA followed by Bonferroni post-test) when drug-treated groups are compared to saline-treated controls. The arrows indicate the moments of each central injection.

Fig. 2 displays typical blood pressure tracings in animals receiving lateral ventricle injections of m-CPBG (panel A), ondansetron (panel B) and ondansetron + m-CPBG (panel C).

Fig. 3 (panel A) shows that naloxone pretreatment (30 nmol) blocks the hypotensive response induced by lateral ventricle injections of m-CPBG. No significant change occurred in HR in any of the groups studied in this experimental set as shown in panel B. Analysis of variance for MAP indicated significant treatment, and significant time effect and treatment × time interactions [$F_{(2,240)} = 14.49$, $p = 0.0003$; $F_{(16,240)} = 1.86$, $p = 0.025$; $F_{(15,240)} = 5.17$, $p < 0.0001$, respectively]. Analysis of variance for HR indicated no time effect, no significant treatment effect, and significant treatment × time interaction [$F_{(16,240)} = 1.11$, $p = 0.3506$; $F_{(2,240)} = 0.56$, $p = 0.5804$; $F_{(15,240)} = 13.05$, $p < 0.0001$, respectively].

In Fig. 4 (panel A) is clear that in animals pretreated with NOR-BNI (10 nmol) the hypotensive response induced by lateral ventricle injections of m-CPBG was suppressed. No change in HR was observed in any of the groups studied in this experimental set, as shown in panel B. Analysis of variance for MAP indicated significant treatment, and significant time effect and treatment × time interactions [$F_{(2,288)} = 22.76$, $p < 0.0001$; $F_{(16,288)} = 6.51$, $p < 0.0001$; $F_{(18,288)} = 9.45$, $p < 0.0001$, respectively]. Analysis of variance for HR indicated significant time effect, no significant treatment effect, and significant treatment × time interaction [$F_{(16,288)} = 2.49$, $p = 0.0014$; $F_{(2,288)} = 0.71$, $p = 0.5035$; $F_{(18,288)} = 18.87$, $p < 0.0001$, respectively].

Fig. 5 (panel A) shows that pretreatment with naltrindole (1 nmol) reverts the hypotensive response achieved by lateral ventricle injections of m-CPBG. Indeed, despite treatment with m-CPBG, animals pretreated with naltrindole rather than experiencing a fall in blood pressure, showed a significant hypertensive response, as compared to saline-treated controls. Yet again, the pharmacological procedures used in this particular experimental set failed to modify HR, as displayed in panel B. Analysis of variance for MAP indicated significant treatment, no significant time effect and significant treatment × time interactions [$F_{(2,304)} = 46.00$, $p < 0.0001$; $F_{(16,304)} = 1.25$, $p = 0.2276$; $F_{(19,304)} = 6.76$, $p < 0.0001$, respectively]. Analysis of variance for HR indicated no time effect, no significant treatment effect, and significant treatment × time interaction [$F_{(16,304)} = 0.66$, $p = 0.8305$; $F_{(2,304)} = 2.44$, $p = 0.1144$; $F_{(19,304)} = 4.99$, $p < 0.0001$, respectively].

Fig. 6 shows that lateral ventricle isolated injections of naloxone, NOR-BNI or naltrindole, in distinct groups of animals, at the same doses used in the previous experimental sets were unable to modify either blood pressure (panel A) or HR (panel B). Analysis of variance for MAP indicated no significant treatment and time effects and significant treatment × time interactions [$F_{(3,400)} = 0.22$, $p = 0.88$; $F_{(16,400)} = 0.92$, $p = 0.5452$; $F_{(25,400)} = 9.54$, $p < 0.0001$, respectively]. Analysis of variance for HR indicated significant time effect, no significant treatment effect, and significant

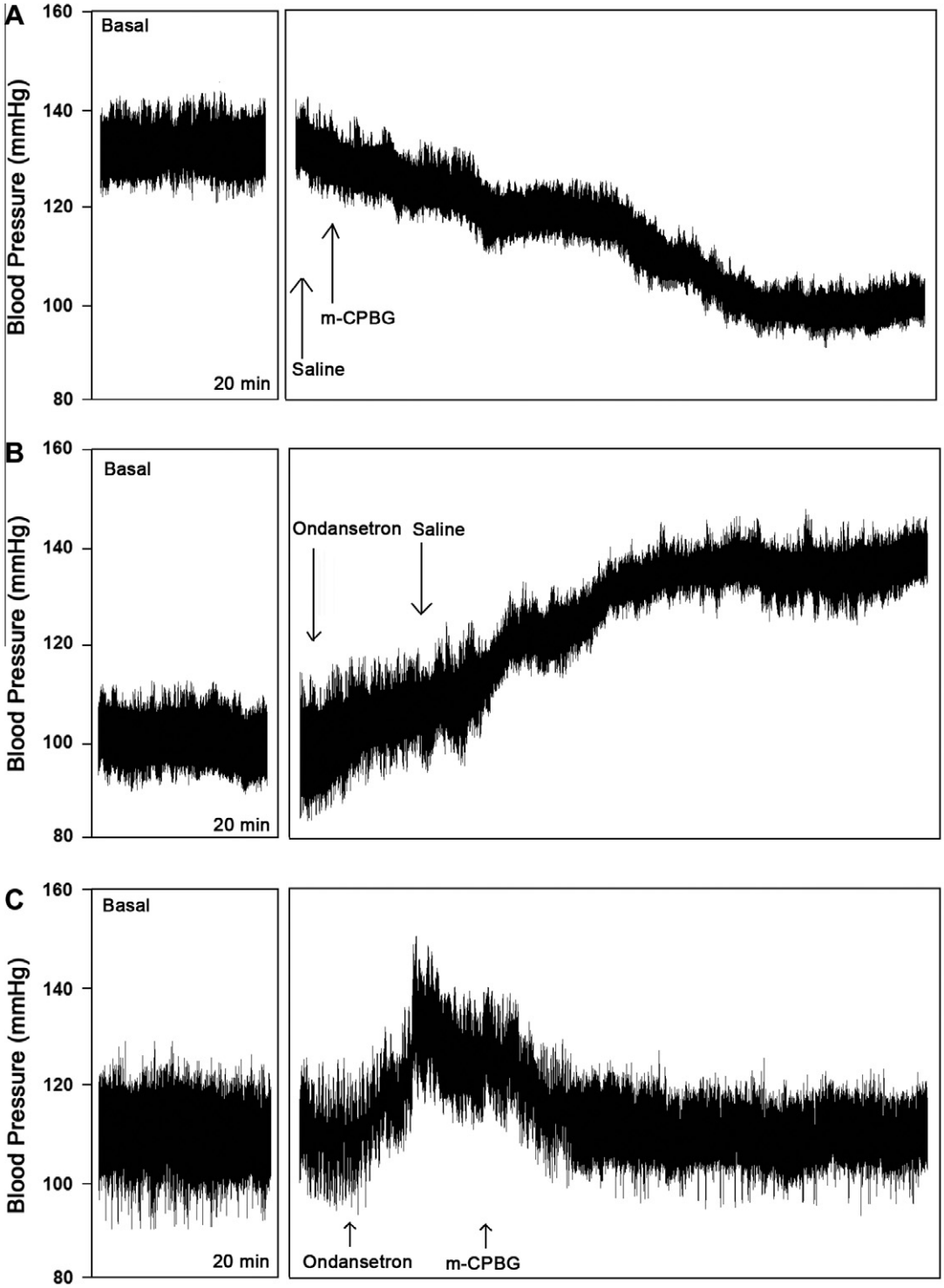

Fig. 2. Typical blood pressure tracings from animals receive saline + m-CPBG (panel A), ondansetron + saline (panel B) and ondansetron + m-CPBG (panel C).

treatment × time interaction [$F_{(16,400)} = 2.21$, $p = 0.0046$; $F_{(3,400)} = 2.26$, $p = 0.1044$; $F_{(25,400)} = 18.26$, $p < 0.0001$, respectively].

4. Discussion

The data obtained in the present study clearly demonstrate that: (1) pharmacological activation of central 5-HT_3 receptors results in a significant decrease in blood pressure; (2) the blockade of those receptors by a selective antagonist induces an acute hypertensive response; (3) the pharmacological blockade of central opioid receptors by three different opioid antagonists exhibiting variable degrees of selectivity to μ, κ and δ opioid receptors always suppressed the hypotensive response induced by central 5-HT_3 receptor stimulation; (4) the blockade of opioid receptors by the same opioid antagonists that impaired the hypotensive effect of central 5-HT_3 receptor stimulation failed to modify blood pressure in animals not submitted to pharmacological manipulations of central 5-HT_3 receptor function.

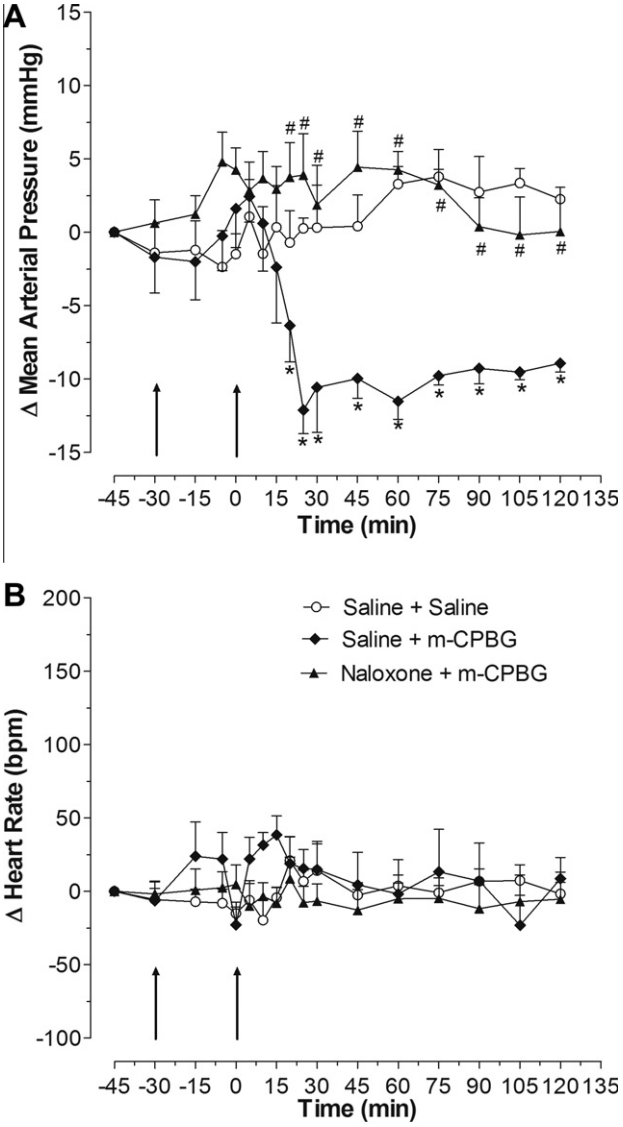

Fig. 3. Changes (Δ) in mean arterial pressure (MAP – Panel A) and heart rate (HR – Panel B) in animals receiving lateral ventricle injections of m-CPBG pretreated with naloxone or saline compared to controls (saline + saline). Data are presented as means ± SEM. The following groups are presented: saline + m-CPBG ($n = 5$; basal MAP = 116.5 ± 1.1; basal HR = 313.4 ± 4.9); naloxone + m-CPBG ($n = 7$; basal MAP = 120.6 ± 1.3; basal HR = 360.9 ± 17.1); saline + saline ($n = 6$; basal MAP = 113.2 ± 0.7; basal HR = 339.3 ± 23.5). *Statistically significant differences (two-way ANOVA followed by Bonferroni post-test) when drug-treated groups are compared to saline-treated controls. The arrows indicate the moments of each central injection. #Statistically significant differences when the group receiving naloxone + m-CPBG is compared to the group of animals receiving saline + m-CPBG. The arrows indicate the moments of each central injection.

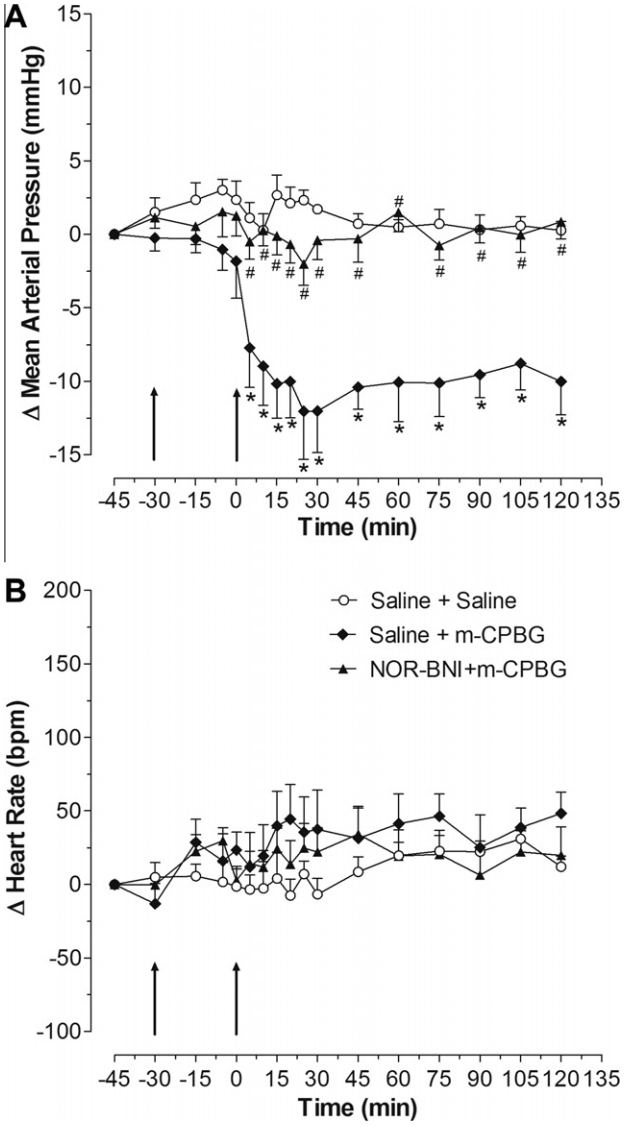

Fig. 4. Changes (Δ) in mean arterial pressure (MAP – Panel A) and heart rate (HR – Panel B) in animals receiving lateral ventricle injections of m-CPBG pretreated with NOR-BNI or saline compared to controls (saline + saline). Data are presented as means ± SEM. The following groups are presented: saline + m-CPBG ($n = 5$; basal MAP = 112.7 ± 4.5; basal HR = 330.3 ± 14.4); NOR-BNI + m-CPBG ($n = 10$; basal MAP = 102.4 ± 2.4; basal HR = 356.2 ± 18.9); saline + saline ($n = 6$; basal MAP = 109.2 ± 3.0; basal HR = 317.0 ± 9.3). *Statistically significant differences (two-way ANOVA followed by Bonferroni post-test) when drug-treated groups are compared to saline-treated controls. The arrows indicate the moments of each central injection. #Statistically significant differences when the group receiving NOR-BNI + m-CPBG is compared to the group of animals receiving saline + m-CPBG. The arrows indicate the moments of each central injection.

Brain 5-HT_3 receptor physiology was selected for this study because: (1) the role of these receptors in the control of neurovegetative phenomena is yet to be fully understood; (2) they clearly participate in several mechanisms related to anxiolytic, antipsychotic and cognitive processes (Costall and Naylor, 1992; Gyermek, 1995; Hagan et al., 1993), making them probable targets for new drugs to be used in humans, and (3) pharmacological manipulations of central 5-HT_3 receptors are already quite common in current clinical practice since 5-HT_3 receptor antagonists are first choice anti-emetic agents in the treatment of nausea and vomiting during chemotherapy (Gregory and Ettinger, 1998).

Comprehensive reviews provide substantial information on the role of brain serotonin in the control of blood pressure. It has been demonstrated that distinct receptors and brain serotonergic regions participate as interacting units performing discrete functions in the central network system involved in the control of cardiovascular activity (Ramage, 2001; Nalivaiko and Sgoifo, 2009). Indeed, brain serotonergic control of vasomotricity and cardiac function may lead to bradycardia or tachycardia, hypotension or hypertension, depending on the brain area studied and the functional status of the different serotonin receptor subtypes (Nalivaiko and Sgoifo, 2009). The importance of brain serotonergic pathways in the control of blood pressure has already been shown. Indeed, we have demonstrated that central 5-HT_3 receptors participate in the control of blood pressure in non-stressed rats, and that the pharmacological activation of

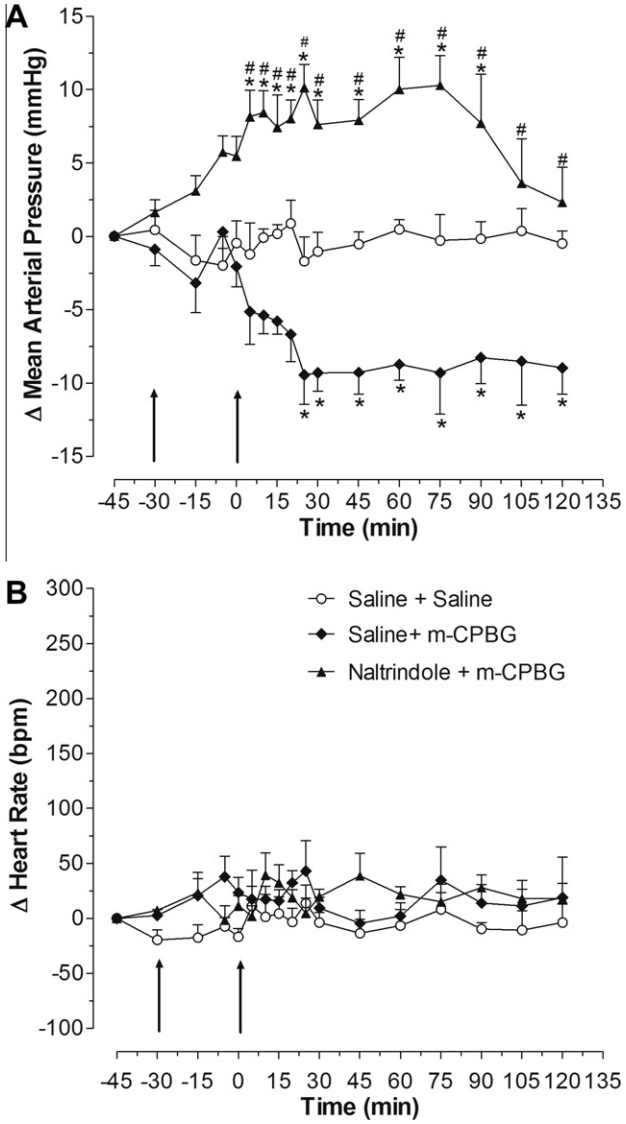

Fig. 5. Changes (Δ) in mean arterial pressure (MAP – Panel A) and heart rate (HR – Panel B) in animals receiving lateral ventricle injections of m-CPBG pretreated with naltrindole or saline compared to controls (saline + saline). Data are presented as means ± SEM. The following groups are presented: saline + m-CPBG (n = 8; basal MAP = 111.0 ± 2.6; basal HR = 353.1 ± 20.3); naltrindole + m-CPBG (n = 8; basal MAP = 113.4 ± 1.8; basal HR = 324.3 ± 11.7); saline + saline (n = 6; basal MAP = 106.5 ± 3.0; basal HR = 304.8 ± 8.5). *Statistically significant differences (two-way ANOVA followed by Bonferroni post-test) when drug-treated groups are compared to saline-treated controls. The arrows indicate the moments of each central injection. #Statistically significant differences when the group receiving naltrindole + m-CPBG is compared to the group of animals receiving saline + m-CPBG. The arrows indicate the moments of each central injection.

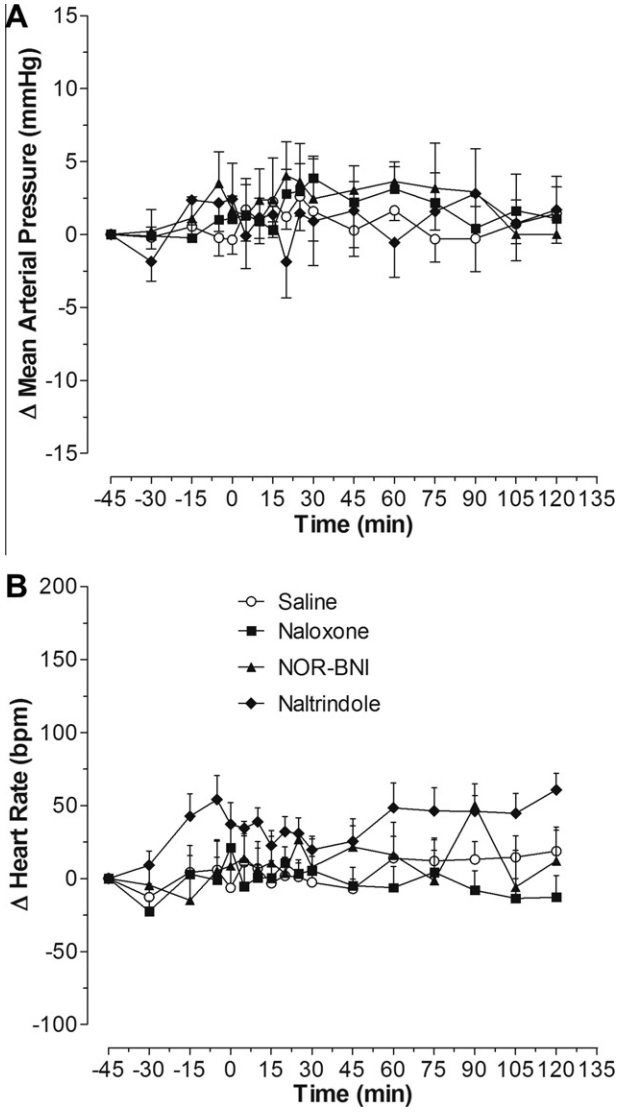

Fig. 6. Changes (Δ) in mean arterial pressure (MAP – Panel A) and heart rate (HR – Panel B) in animals receiving lateral ventricle injections of different opioid antagonists or saline. Data are presented as means ± SEM. The following groups are presented: naloxone (n = 10; basal MAP = 110.7 ± 2.5; basal HR = 341.8 ± 11.7); NOR-BNI (n = 6; basal MAP = 108.2 ± 2.8; basal HR = 325.2 ± 16.3); naltrindole (n = 6; basal MAP = 108.2 ± 2.2; basal HR = 301.1 ± 14.3); saline (n = 7; basal MAP = 112.0 ± 2.5; basal HR = 319.1 ± 12.0).

central 5-HT_3 receptors by selective agonists blocks stress-induced hypertension (Ferreira et al., 2004). Additionally, we have shown that 5-HT_3 receptors located at the MS/vDB tonically inhibit sympathetic activity through an action mediated by angiotensinergic mechanisms in that same area (Urzedo-Rodrigues et al., 2011). The present study, confirm that a 5-HT_3 receptor-dependent mechanism seems to be part of the brain serotonergic system that contributes to cardiovascular regulation since the hypertensive response observed after ondansetron administration indicates that central 5-HT_3 receptors exert a tonic inhibitory drive on blood pressure.

It has been clearly established that brain opioid pathways participate in cardiovascular control. The cardiorespiratory effects of alkaloid opioids such as morphine were evident since the second half of the 18th century (Feuerstein and Sirén, 1987) and opioid peptides and their receptors are found in brain areas involved in cardiovascular control (Fichna et al., 2007; George et al., 1994). However, a review of the literature brings to light a complex picture, in which distinct opioid receptor subtypes and discrete brain opioid circuitries play an integrated role in cardiovascular regulation (Bodnar, 2009; Vaccarino and Kastin, 2001). Pharmacological manipulations of central opioid pathways yield both hypertensive and hypotensive effects. Lateral ventricle injections of endogenous opioid peptides may induce significant pressor responses (Lang et al., 1982) and intracerebroventricular administration of β-endorphin increases blood pressure in obese rats (Ku, 2006). On the other hand, cerebroventricular injections of β-endorphin lead to a significant fall in blood pressure (Laubie et al., 1977) and the same peptide injected into the hypothalamic preoptic area abolished the pressor response induced by subcutaneous administration of hypertonic saline solution, and causes hypotension and bradycardia (Caeiro and Vivas, 2008). Furthermore, microinjections

of several opioid receptor agonists into the rostral ventral lateral medulla (RVLM) produce marked hypotension (Guyenet et al., 2002). Additionally, extensive anatomofunctional connections among opioidergic pathways and brain sites involved in cardiovascular control have been demonstrated (Ross et al., 1985; Mezey et al., 1987).

In the present study, we investigated the possible interactions between central serotonergic and opioidergic pathways in blood pressure control. The data obtained here seem to indicate that brain opioid pathways play a crucial role in the genesis of the hypotensive response observed after central 5-HT_3 receptor stimulation, and that three distinct opioid receptor subtypes (μ, κ and δ) may participate in this phenomenon. This suggests a functional interaction between two distinct brain circuitries involved in cardiovascular control (serotonergic and opioidergic).

The above mentioned interaction between serotonergic and opioidergic pathways in the central nervous system has been widely documented. It has been demonstrated that opioid release in the spinal cord is modulated by 5-HT_{1A} receptors (Song et al., 2007) and that 5-HT_{1A} receptor mRNA co-localizes with enkephalin in the dorsal horn (Zhang et al., 2002). Serotonin and some selective serotonin reuptake inhibitors may exert a significant antinociceptive effect that seems to be mediated by opioids (Nozaki and Kamei, 2006). Naloxone blocks or attenuates the analgesic effect of serotonin or serotonergic agonists, indicating that endogenous opioids might be involved in serotonin-induced antinociception (Kwiat and Basbaum, 1992; Fields and Basbaum, 1994). Additionally, *p*-chlorophenylalanine, a neurotoxic agent that selectively destroys brain serotonergic pathways, decreases dynorphin levels (Sharma et al., 1992) and pro-dynorphyn mRNA in different brain areas (D'Addario et al., 2007). Finally, behavioral patterns induced by the stimulation of brain 5-HT_2 receptors seem to be opioid-mediated (Corne et al., 1963; Vetulani et al., 1980) and 5-HT_3 receptor antagonists such as ondansetron reduce opioid withdrawal behaviors both in mice and in human beings (Chu et al., 2009). Furthermore, it is interesting to note that both serotonergic and opioidergic systems are simultaneously activated in the central nervous system during stress (Lanfumey et al., 2008; Chaouloff et al., 1999; Bruchas et al., 2010; Drolet et al., 2001).

The present results suggest that the interaction between serotonergic and opioidergic pathways in the brain may be important in maintaining blood pressure within the normal range. Since different studies show that serotonin may modulate the neuronal release of opioid, it is reasonable to suggest that the hypotensive effect induced by activation of the 5-HT_3 receptors depends on the downstream release of the opioid. The μ-, κ- and δ-opioid receptors are coupled to adenylyl cyclase, K^+ channels, and voltage-activated Ca^{2+} channels (Childers, 1991). These receptors have been identified in the cell body, as well as in the axon terminals and their activation at synaptic terminals may change the spike duration controlling Ca^{2+} influx and, therefore, inhibiting neurotransmitter release (Sesack and Picke, 1992; Schoffelmeer et al., 1992a,b). It is possible that the release of opioid peptides induced by the activation of the 5-HT_3 receptors may inhibit the release of the neurotransmitters that control sympathetic tonus and normal blood pressure. However, in the present study the intrinsic, cellular mechanism by which 5-HT_3 and opioid receptors interact cannot be established with the experimental protocols used.

All three opioid receptors studied here seem to be crucial for the fall in blood pressure that follows central 5-HT_3 receptor stimulation, and each one of them individually participate in central cardiovascular regulation. Stimulation of central μ-opioid receptors by intracerebroventricular injections of selective μ-opioid agonists such as morphine, β-endorphin and DAMGO induces hypotension in a variety of species (Holaday, 1983; Johnson et al., 1985; Unal et al., 1997; Champion et al., 1998; Olson et al., 1998; Vaccarino et al., 1999). Also, central κ-opioid receptors mediate cardiovascular activity since injections of dynorphyn, an endogenous opioid with high affinity for κ-opioid receptors, and non-peptide κ-opioid receptor agonists on rat hippocampus induce a significant decrease in blood pressure in rats (Pugsley, 2002; McConnaughey et al., 1998; Shen and Ingenito, 1999). Pharmacological stimulation of κ-opioid receptors located at the nucleus of the solitary tract induces a significant hypotensive response in rats (Keay et al., 1997) and intracerebroventricular injections of κ-opioid receptor agonists are consistently associated with a decrease in blood pressure in rats (Rao et al., 2003). Furthermore, stimulation of δ-opioid receptors located in the hypothalamus (Faden and Feuerstein, 1983), in the nucleus of the solitary tract (Feldman et al., 1996) and in the rostral ventrolateral medulla (Morilak et al., 1990) induces a significant decrease in blood pressure. Additionally, activation of δ-opioid receptors in rat ventrolateral medulla inhibits somatosympathetic reflexes (Miyawaki et al., 2002) and hypotension induced by endotoxic shock or hemorrhage seems to be mediated by central δ-opioid receptors (D'Amato and Holaday, 1984; Henderson et al., 2002; Cavun et al., 2001; Frithiof et al., 2007).

Opioid pharmacology is a rather complex matter and studies employing pharmacological tools to block or to stimulate opioid function have to take into consideration the characteristic profiles of the individualdrugs used. However, in the present study the opiatergic antagonists used are the most suitable agents currently employed in pharmacological protocols tailored to explore functional aspects of opioid receptors. The antagonistic effect of naloxone on μ-opioid receptors is greater than its antagonistic effect on other opioid receptor subtypes, and the compound is normally considered a preferential μ-opioid receptor antagonist (Zimmerman and Leander, 1990). NOR-BNI is an opioid receptor antagonist with preferential κ-opioid receptor antagonistic activity (Portoghese et al., 1987; Takemori et al., 1988) and naltrindole is one of the most potent δ-opioid receptors antagonist available (Portoghese, 1993; Portoghese et al., 1990). Therefore, it is reasonable to assume that the absence of a hypotensive response after the stimulation of central 5-HT_3 receptors when μ, κ and δ opioid receptors are independently blocked indicates that each one of these receptors is essential for the expression of hypotension in these particular circumstances. Furthermore, simultaneous activation of μ, κ and δ opioid receptors appears to be necessary for 5-HT_3 receptor-dependent hypotension to occur since the blockade of each one of these receptors completely abolishes this effect.

The blockade of μ and κ opioid receptors impaired the hypotensive response observed after central 5-HT_3 receptor stimulation. However, animals pretreated with naltrindole, a preferential δ-opioid receptor antagonist, showed not only a reversion of the hypotension seen when 5-HT_3 receptors are stimulated but presented a significant hypertensive response. This may mean that during central 5-HT_3 receptor stimulation, central δ-opioid receptors exert a tonic, negative drive on blood pressure. This tonic inhibitory drive exerted by δ-opioid receptors seems to be restricted to animals in which central 5-HT_3 receptors are stimulated since the administration of naltrindole alone has no effect on animals in which central 5-HT_3 receptors are not pharmacologically activated. Furthermore, in animals in which central 5-HT_3 receptors are pharmacologically stimulated, this tonic, inhibitory drive that is dependent on μ and κ opioid receptors is not observed. The pattern of opioid receptors distribution in the brain is distinct for each receptor subtype. In addition the density of the opioid receptors varies greatly in the different brain regions (Sharif and Hughes, 1989; Mansour et al., 1995; Kitchen et al., 1997). These anatomic differences among the opioid receptors subtypes may account for their functional diversity. Moreover, it is important to note that, in the absence of

central 5-HT_3 receptor stimulation, none of the opioid antagonists was capable of altering blood pressure, indicating that the reduction in endogenous opioid activity promoted by these drugs, at the doses used, was unable to affect central blood pressure regulation.

We have previously demonstrated that the blockade and the stimulation of central 5-HT_3 receptors impair baroreflex activity. Indeed, no tachycardic response is observed after the hypotension that follows the stimulation of central 5-HT_3 receptors by m-CPBG and no bradycardia is seen during hypertension that follows the blockade of central 5-HT_3 receptors by ondansetron (Ferreira et al., 2004). The same phenomenon is observed here. There is no compensatory tachycardia in hypotensive animals after central 5-HT_3 receptor stimulation by m-CPBG. Also, in the group of animals receiving m-CPBG but pretreated with naltrindole hypotension was reverted and a hypertensive response was apparent without any associated bradycardia.

In the present paper, it was decided to study the effects of pharmacological manipulations on central 5-HT_3 receptors and opioid receptors by injecting the drugs intracerebroventricularly instead of studying the effect of the drugs in any particular region of the brain. The reason for this choice is that the numerous pharmacological serotonergic and opiatergic agents used in clinical therapeutics are systemically administered and reach the brain as a whole. The approach selected for this study is, therefore, suitable for investigating the cardiovascular effects generated by these agents through their action on the central nervous system alone, excluding the myriad of effects that would result from their interaction with peripheral receptors. However, this experimental protocol does not permit identification of the specific brain areas involved in the responses observed here. Further studies should be performed to clarify the interaction between 5-HT_3 receptors and mu, kappa and delta opioid receptors in specific brain areas on the control of blood pressure.

In summary, the data obtained here indicate that a 5-HT_3 receptor-dependent mechanism seems to be part of the brain serotonergic system that contributes to cardiovascular regulation since the hypertensive response observed after ondansetron administration indicates that central 5-HT_3 receptors exert a tonic inhibitory drive on blood pressure. Furthermore, the present data clearly indicate that the hypotensive response observed after pharmacological stimulation of central 5-HT_3 receptors depends on the functional integrity of brain μ, κ and δ opioid receptors, suggesting that a functional interaction between serotonergic and opiatergic pathways in the brain is part of the complex, multifactorial system that regulates blood pressure in the central nervous system.

Acknowledgments

We are grateful to Mr. José de Souza and Mr. Edison Brandão for their skillful technical assistance. The present work was supported by grants provided by the Brazilian Council of Research (CNPq), #304116/2006-8 and 304.117/2006-4, and by the Financial Agency for the Support of Research of the State of Bahia (FAPESB).

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