Available online at www.sciencedirect.com
SCIENCE DIRECT
Brain Research 981 (2003) 151–159
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Research report

Central 5-$HT_{2B/2C}$ and 5-HT_3 receptor stimulation decreases salt intake in sodium-depleted rats

Letícia Castro[a], Rodrigo Athanazio[a], Marcelo Barbetta[a], Ana Cláudia Ramos[a], Ana Luiza Angelo[a], Igor Campos[a], Bruno Varjão[a], Hilda Ferreira[b], Josmara Fregoneze[a], Emilio de Castro e Silva[a,*]

[a]*Department of Physiology, Health Sciences Institute, Federal University of Bahia, 40110-100 Salvador—Bahia, Brazil*
[b]*Life Sciences Department, Bahia State University, 41195-001 Salvador—Bahia, Brazil*

Accepted 14 May 2003

Abstract

In the present study, we investigated the participation of central 5-$HT_{2B/2C}$ and 5-HT_3 receptors in the salt intake induced by sodium depletion in Wistar male rats. Sodium depletion was produced by the administration of furosemide associated with a low salt diet. Third ventricle injections of mCPP, a 5-$HT_{2B/2C}$ agonist, at doses of 80, 160 and 240 nmol, promoted a dose-dependent reduction in salt intake in sodium-depleted rats. The inhibitory effect produced by central administration of mCPP was abolished by the central pretreatment with SDZ SER 082, a 5-$HT_{2B/2C}$ antagonist. Similar results were obtained with third ventricle injections of m-CPBG (80, 160 and 240 nmol), a selective 5-HT_3 agonist that also induced a dose-related decrease in salt intake in sodium-depleted rats. The central pretreatment with LY-278,584, a selective 5-HT_3 receptor antagonist, was able to impair the salt intake inhibition elicited by third ventricle injections of m-CPBG. Central administration of each one of the antagonists alone or a combination of both antagonists together did not significantly change salt intake after sodium depletion. On the other hand, the central administration of both mCPP and m-CPBG, in the highest dose used to test their effect on salt intake (240 nmol), was unable to modify blood pressure in sodium-depleted rats. It is concluded that: (1) pharmacological activation of central 5-$HT_{2B/2C}$ and 5-HT_3 receptors diminishes salt intake during sodium depletion, (2) an inhibitory endogenous drive exerted by central 5-$HT_{2B/2C}$ and 5-HT_3 receptors does not seem to exist and (3) the reduction in salt intake generated by the pharmacological activation of these central receptors is not produced by an acute hypertensive response.

Theme: Neural basis of behavior

Topic: Ingestive behaviors

Keywords: Sodium appetite; Serotonin; Sodium depletion

1. Introduction

Osmolarity and blood pressure control in mammals is achieved by the action of multiple regulatory loops inducing endocrine, nervous and behavioral effects designed to maintain those parameters within their narrow physiological ranges [4].

Sodium homeostasis, a necessary step in the control of both osmolarity and blood pressure, results from very fine regulatory actions adjusting the intake/excretion balance of water and sodium. The sodium intake/excretion balance is mainly dependent on two complementary parameters—sodium appetite and renal sodium excretion—that are strongly controlled by the central nervous system [20].

Brain serotonin circuitries are clearly involved in the control of sodium appetite, the crucial motivation inducing sodium intake. Indeed, serotonin pathways in lateral parabrachial nucleus seem to exert a tonic inhibition in salt intake induced by sodium depletion [24] or by the stimulation of prosencephalic angiotensinergic pathways [11].

*Corresponding author. Tel.: +55-71-235-7518; fax: +55-71-337-0591.
E-mail address: emilio@ufba.br (E. de Castro e Silva).

0006-8993/03/$ – see front matter
doi:10.1016/S0006-8993(03)03015-4

Peripheral administration of both fenfluramine, a serotonin uptake inhibitor and releaser, and MK-212, a 5-HT_{2C} receptor agonist, inhibits salt intake [25]. Also, peripheral and central administration of ketanserin, a 5-HT_2 receptor antagonist, decreases salt intake induced by mineralocorticoid stimulation [17].

The same brain areas that stimulate salt intake generally increase water intake, while central structures decreasing sodium appetite normally inhibit thirst generated both by physiological and pharmacological stimuli. In previous papers, we have clearly demonstrated that the pharmacological stimulation of several brain serotonin receptors significantly decreases water intake [6–8,14].

As a next step in our investigation, in this study we explore whether the pharmacological stimulation of 5-HT_3 and 5-$HT_{2B/2C}$ receptors is able to modify salt intake in sodium-depleted rats.

2. Method

2.1. Animals

Wistar male rats weighing 240±20 g, housed in individual cages and kept under controlled light (lights on from 7:00 a.m. to 7:00 p.m.) and temperature (22–24 °C) conditions, were used in this study.

2.2. Surgical procedures

Five days before the experimental sessions, the third ventricle was cannulated under pentobarbital anesthesia (50 mg/kg i.p.). This was carried out by implanting a 22-gauge stainless steel cannula (15 mm in length) using a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). The following coordinates were used: anteroposterior=0.5 mm behind bregma; lateral=0.0 mm; vertical 8.5 mm below the skull. The animals were placed in the stereotaxic apparatus with the head inclined +0.2 mm upward, avoiding lesions to the midline structures related to body fluid and electrolyte control. The cannulas were cemented to the skull bone with dental acrylic and an obturator (28 gauge) was provided to avoid obstruction. To confirm whether the tip of the cannula was in the proper place, the animals were sacrificed by CO_2 inhalation and a third ventricle injection of Blue Evans dye was carried out. Only data from animals in which the cannulas were strictly inside the third ventricle were analyzed.

2.3. Drugs and microinjections

The following drugs were used: *m*-chlorophenylbiguanide hydrochloride (1-(3-chlorophenyl)biguanide; m-CPBG), a selective 5-HT_3 agonist [27,30], mCPP (1-(3-Chlorophenyl)piperazine), a 5-$HT_{2B/2C}$ agonist [21], and SDZ SER 082 [(+)-*cis*-4,5,7a,8,9,10,11,11a-octahydro-7H-10-methylindolo[1,7-*bc*][2,6]-naphthyridine], a 5-$HT_{2B/2C}$ receptor antagonist [32], were purchased from Tocris Cookson (Ballwin, MO, USA). LY-278,584 (1-methyl-*N*-[8-methyl-8-azabicyclo(3.2.1)-oct-3-yl]-1H-indazole-3-carboxamide), a selective 5-HT_3 receptor antagonist [1,16] was acquired from Sigma (St Louis, MO), USA. Furosemide, a loop diuretic, was purchased from Aventis Pharma Ltd., São Paulo, Brazil. All drugs were dissolved in isotonic saline solution. Third ventricle injections were achieved 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. Sodium depletion

Animals in the sodium depletion protocol had simultaneous access to two bottles (distilled water and 1.5% saline solution) and standard rat chow from the period immediately after third ventricle cannulation until the moment of furosemide administration. Sodium depletion was achieved by two subcutaneous administrations of furosemide (10 mg/kg). These two injections were given 24 and 2 h before the experimental sessions. Immediately after the first furosemide injection, the access to 1.5% saline was halted. The animals continued to have free access to distilled water, and normal rat chow was replaced by a low sodium diet (0.001% Na^+ and 0.33% K^+). Animals receiving two subcutaneous injections of isotonic saline solution instead of furosemide in the same schedule, and having access to standard chow were used as control group.

2.5. Plasma and urinary sodium measurement

The determination of both urinary and plasma sodium was achieved by flame photometry, using a digital flame photometer (Micronal, model B262, São Paulo, Brazil).

2.6. Blood pressure recording

To record blood pressure, a carotid catheter was connected to a pressure transducer (Hewlett-Packard, model 21080A) whose signal was amplified and digitally recorded by an analog-to-digital interface (AqDados, version 5, Lynx Tecnologia Eletrônica Ltda, São Paulo, Brazil) and recorded (1 kHz) on a microcomputer (IBM/PC-AT 586) for later analysis.

2.7. Experimental design

After surgery, the animals in all study groups had free access to two different bottles: one containing distilled water and the other containing 1.5% saline solution. The animals were manipulated every day in order to minimize the stress of the experimental maneuvers.

To test the participation of central 5-$HT_{2B/2C}$ and 5-HT_3 receptors in salt intake, distinct groups of sodium-depleted animals received third ventricle injections of different doses (80, 160 and 240 nmol) of 5-$HT_{2B/2C}$ or 5-HT_3 agonists (mCPP and m-CPBG, respectively). Control sodium-depleted animals received third ventricle injections of isotonic saline solution. Thirty minutes after the third ventricle injections, the bottles containing 1.5% saline solution were reintroduced into the cages and both water and salt intakes were recorded for the next 120 min. To test the specificity of the pharmacological agents employed, different groups of animals receiving the highest dose of mCPP or m-CPBG were pretreated with selective antagonists 10 min before receiving those agonists. SDZ SER 082 (240 nmol) and LY-278,584 (120 nmol) were used as 5-$HT_{2B/2C}$ and 5-HT_3 antagonists, respectively. To analyze the effect of these two antagonists when each one of them was administered alone, distinct groups of animals received third ventricle injections of SDZ SER 082 (240 nmol) and LY-278,584 (120 nmol) 40 min before the access to 1.5% saline solution was re-established, and had their water and salt intakes recorded for 120 min. To ascertain whether the simultaneous blockade of both 5-HT_3 and 5-$HT_{2B/2C}$ central receptors modifies salt intake in sodium-depleted rats in a way that would differ from that observed when each antagonist was employed alone, we administered the two antagonists together to a distinct group of furosemide-treated animals and recorded their salt intake as for the other groups.

To test the effect of furosemide administration on urinary sodium excretion and plasma sodium concentration, a distinct group of animals were submitted to the same sodium depletion protocol previously mentioned. They were placed in stainless steel metabolic cages and had urine output collected for 24 h for determination of renal sodium excretion. Immediately after this period, aortic blood samples were collected under surgery for determination of plasma sodium concentration, after centrifugation.

To investigate if third ventricle injections of mCPP could produce illness-like effects that could interfere in a nonspecific way with salt intake, we submitted a distinct group of animals to an aversion test in which a temporal association between the novel taste of a 0.25% saccharin solution and the sensation of discomfort induced by lithium chloride administration was established by the animals. A complete description of this test is given in detail elsewhere [6].

To test the effects of the administration of 5-$HT_{2B/2C}$ and 5-HT_3 agonists on blood pressure, distinct groups of sodium-depleted animals, whose blood pressure had already been monitored for 10 min, received third ventricle injections of mCPP or m-CPBG (240 nmol in each case). In each of those groups, blood pressure continued to be recorded for the next 150 min after the pharmacological agents were injected. The experimental protocol used in this case was identical to that used to study salt intake in the previous groups.

All experimental protocols were conducted according to the regulations suggested by the National Institutes of Health (USA).

2.8. Statistical analysis

A computer software package (SigmaStat for Windows, Jandel Scientific, San Rafael, CA, USA) was used to carry out one-way analysis of variance at each time period. The post-hoc Student–Newman–Keuls test was used for comparison of each treatment to its corresponding time in the control groups. The Student's *t*-test was used to analyze the data concerning the aversion test and the effects of furosemide on urine flow, renal sodium excretion and natremia. The data are presented as mean±S.E.M. The groups were considered significantly different when $P < 0.05$.

3. Results

Fig. 1 (panel A) displays the effects of third ventricle injections of mCPP, a 5-$HT_{2B/2C}$ agonist, on salt intake in sodium-depleted animals. As expected, control sodium-depleted animals receiving third ventricle injections of vehicle (depl+vehicle) presented a significant increase in salt intake, compared to the control group of animals receiving third ventricle injections of vehicle but not submitted to sodium depletion (no-depl+vehicle). At the lowest dose employed (80 nmol), mCPP did not affect the high salt intake exhibited by control sodium-depleted rats (depl+vehicle). At the intermediate dose (160 nmol) and at the highest dose employed (240 nmol), mCPP exerted a significant inhibition of salt intake that is observed throughout the experiment, compared to the same control group.

Fig. 1 (panel B) shows the effect of third ventricle injections of mCPP, a 5-$HT_{2B/2C}$ agonist, on water intake in sodium-depleted animals. There were no statistically significant differences among groups.

Fig. 2 (panel A) shows the effect of pretreatment with SDZ SER 082, a 5-$HT_{2B/2C}$ antagonist, on salt intake in sodium-depleted rats treated with mCPP. Here, control sodium-depleted animals pretreated with vehicle receiving 240 nmol of mCPP (depl+vehicle+mCPP) present a significant reduction in salt intake, compared to animals pretreated and treated with vehicle (depl+vehicle+vehicle). Sodium-depleted animals treated with the same dose of mCPP but pretreated with 240 nmol of SDZ SER 082 (depl+SDZ+mCPP) showed a salt intake that is not significantly different from that exhibited by control sodium-depleted animals pretreated and treated with vehicle (depl+vehicle+vehicle).

Fig. 2 (panel B) shows the effect of pretreatment with

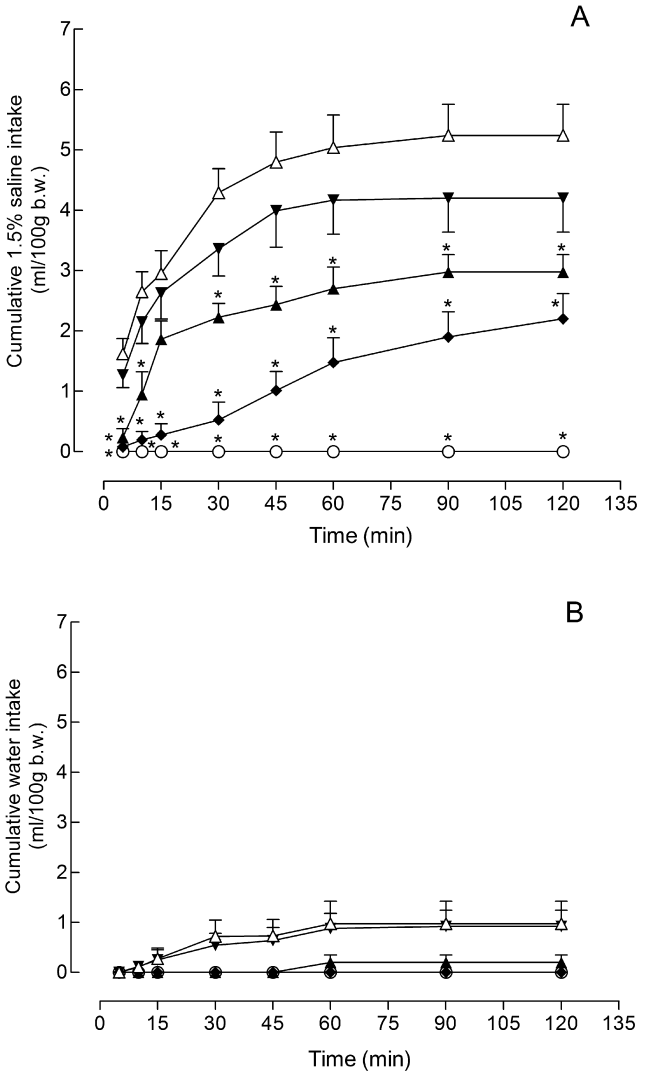

Fig. 1. Cumulative salt (panel A) and water (panel B) intakes (ml/100 g body weight) of sodium-depleted animals treated with third ventricle injections of mCPP, a 5-$HT_{2B/2C}$ agonist, at various doses. The following groups are presented: Vehicle (△; n=11); mCPP 80 nmol/rat (▼ n=9); mCPP 160 nmol/rat (▲; n=9); mCPP 240 nmol/rat (♦; n=11). An additional control group of animals not submitted to sodium depletion and receiving third ventricle injections of vehicle is also shown (○; n=8). Data are presented as mean±S.E.M. Asterisks indicate a statistically significant difference (P<0.05) when the distinct groups are compared to sodium-depleted animals receiving vehicle. Each curve in the graphs represents data obtained with a naïve group of animals.

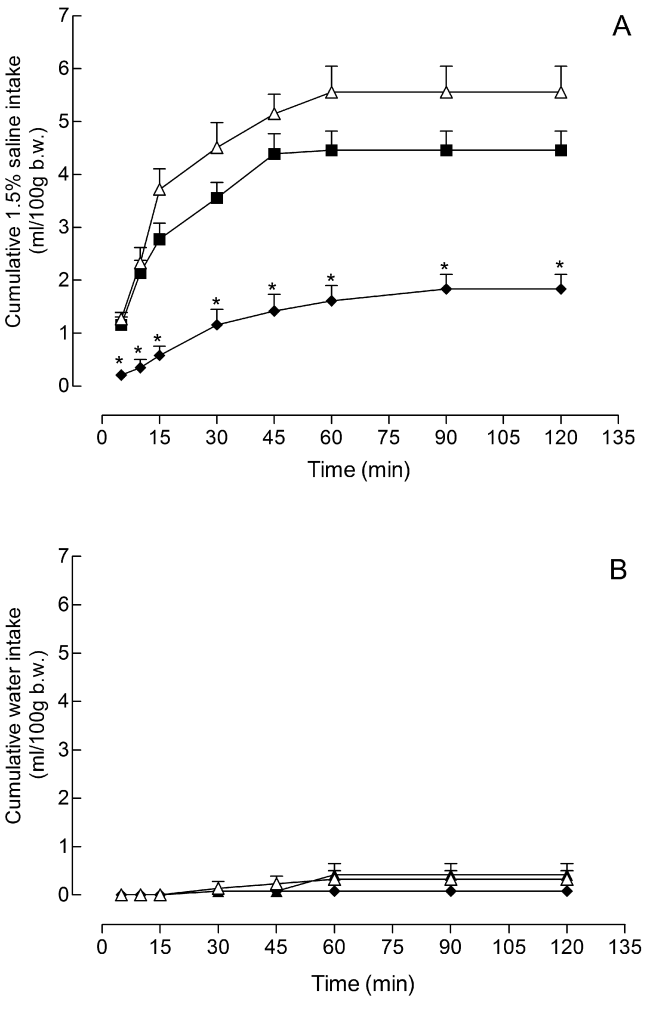

Fig. 2. Cumulative salt (panel A) and water (panel B) intakes (ml/100 g body weight) of sodium-depleted animals treated with third ventricle injections of mCPP (240 nmol) or vehicle but pretreated with third ventricle injections of SDZ SER 082 (240 nmol) or vehicle. The following groups are presented: vehicle+vehicle (△; n=9); vehicle+mCPP (♦; n=10); SDZ SER 082+mCPP (■; n=9). Data are presented as mean±S.E.M. Asterisks indicate a statistically significant difference (P<0.05) when the distinct groups are compared to sodium-depleted animals receiving vehicle. Each curve in the graphs represents data obtained with a naïve group of animals.

SDZ SER 082, a 5-$HT_{2B/2C}$ antagonist, on water intake in sodium-depleted rats treated with mCPP. There were no statistically significant differences among groups.

Fig. 3 (panel A) depicts the effects of third ventricle injections of m-CPBG, a selective 5-HT_3 agonist, on salt intake in sodium-depleted animals. As expected, control sodium-depleted animals receiving third ventricle injections of vehicle (depl+vehicle) displayed a significant increase in salt intake, compared to the control group of animals receiving third ventricle injections of vehicle but not submitted to sodium depletion (no-depl+vehicle). At the lowest dose employed (80 nmol), m-CPBG did not affect the high salt intake exhibited by control sodium-depleted rats (depl+vehicle). Compared to this same control group, m-CPBG at the intermediate dose (160 nmol) or at the highest dose employed (240 nmol) yielded a significant inhibition of salt intake that is seen throughout the experiment.

Fig. 3 (panel B) shows the effect of third ventricle injections of m-CPBG, a selective 5-HT_3 agonist, on water intake in sodium-depleted animals. There were no statistically significant differences among the groups included here.

Fig. 4 (panel A) depicts the effect of pretreatment with LY-278,584, a selective 5-HT_3 antagonist, on salt intake in

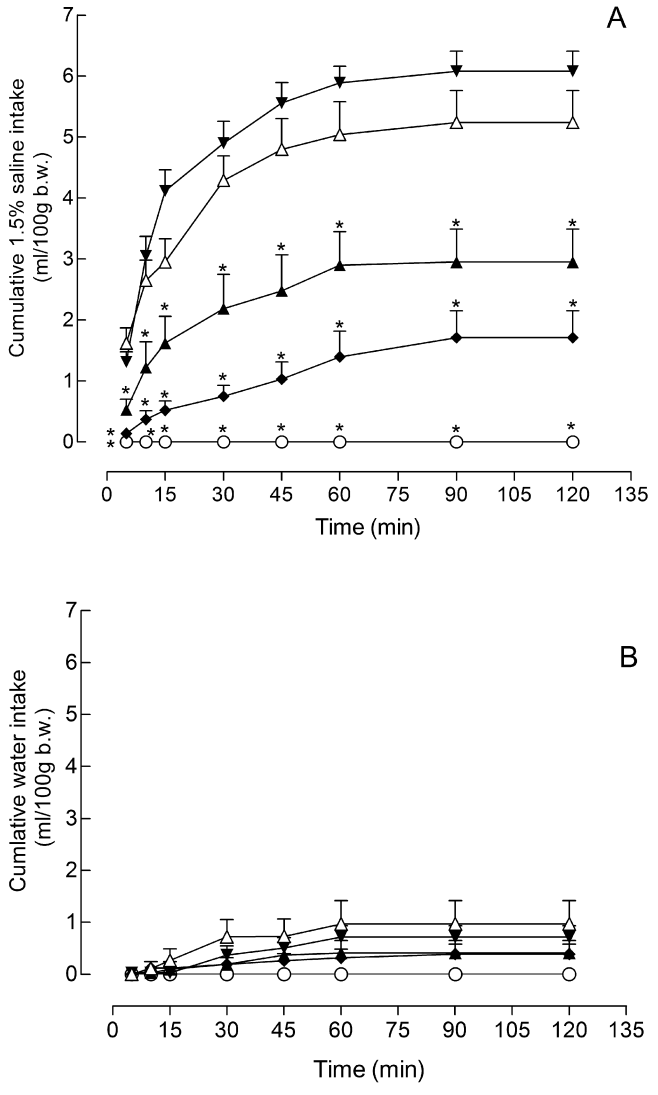

Fig. 3. Cumulative salt (panel A) and water (panel B) intakes (ml/100 g body weight) of sodium-depleted animals treated with third ventricle injections of m-CPBG, a 5-HT_3 agonist, at various doses. The following groups are presented: vehicle (△; n=11); m-CPBG 80 nmol/rat (▼; n=11); m-CPBG 160 nmol/rat (▲; n=10); m-CPBG 240 nmol/rat (♦; n=13). An additional control group of animals not submitted to sodium depletion and receiving third ventricle injections of vehicle is also shown (○; n=8). Data are presented as mean±S.E.M. Asterisks indicate a statistically significant difference (P<0.05) when the distinct groups are compared to sodium-depleted animals receiving vehicle. Each curve in the graphs represents data obtained with a naïve group of animals.

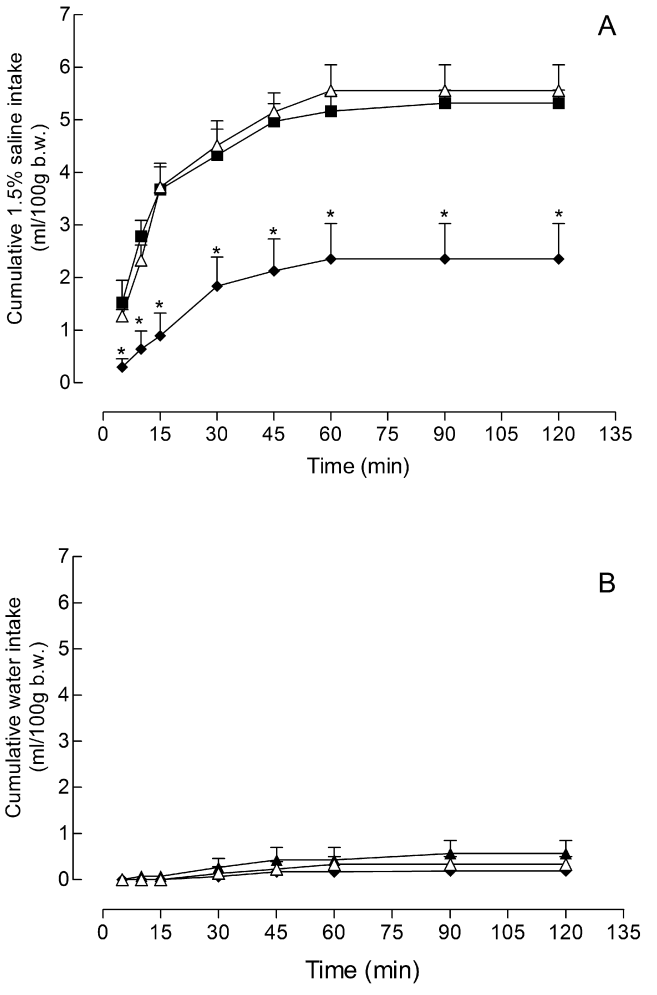

Fig. 4. Cumulative salt (panel A) and water (panel B) intakes (ml/100 g body weight) of sodium-depleted animals treated with third ventricle injections of m-CPBG (240 nmol) or vehicle but pretreated with third ventricle injections of LY-278,584 (120 nmol) or vehicle. The following groups are presented: vehicle+vehicle (△; n=9); vehicle+m-CPBG (♦; n=10); LY-278,584+mCPP (■; n=11). Data are presented as mean±S.E.M. Asterisks indicate a statistically significant difference (P< 0.05) when the distinct groups are compared to sodium-depleted animals receiving vehicle. Each curve in the graphs represents data obtained with a naïve group of animals.

sodium-depleted rats treated with m-CPBG. Here, control sodium-depleted animals pretreated with vehicle receiving 240 nmol of m-CPBG (depl+vehicle+m-CPBG) display a significant reduction in salt intake, compared to animals pretreated and treated with vehicle (depl+vehicle+ vehicle). Sodium-depleted animals treated with the same dose of m-CPBG but pretreated with 120 nmol of LY-278,584 (depl+LY+m-CPBG) showed a salt intake that was not significantly different from that exhibited by sodium-depleted animals pretreated and treated with vehicle (depl+vehicle+vehicle).

Fig. 4 (panel B) shows the effect of pretreatment with LY-278,584, a selective 5-HT_3 antagonist, on water intake in sodium-depleted rats treated with m-CPBG. There were no statistically significant differences among the groups presented here.

Table 1 shows the effect of third ventricle injections of SDZ SER 082 (240 nmol), LY-278,584 (120 nmol) or a combined administration of both antagonists (each one in the same dose mentioned above) on water and salt intake in sodium-depleted rats. There were no statistically significant differences among groups.

Fig. 5 shows that the inhibition of salt intake in sodium-depleted rats after third ventricle injections is not dependent on any illness-like effect. Indeed, the group of rats

Table 1
Cumulative salt and water intake (ml/100 g body weight) of sodium-depleted rats receiving third ventricle injections of LY-278,584 (120 nmol), SDZ SER 082 (240 nmol), a combined injection of both antagonists (each one at the same dose used when injected alone) or vehicle

Intake	Treatment (*n*)	Time (min)				
		15	30	60	90	120
Salt	Saline (11)	3.72±0.38	4.51±0.47	5.56±0.49	5.56±0.49	5.56±0.49
	SDZ Ser 082 (10)	4.22±0.47	5.04±0.54	5.19±0.55	5.56±0.59	5.56±0.59
	LY 278,584 (10)	3.88±0.36	4.53±0.34	4.98±0.32	4.98±0.32	4.98±0.32
	SDZ Ser 082+LY 278,584 (12)	3.21±0.22	4.38±0.27	5.69±0.48	6.13±0.40	6.13±0.40
Water	Saline (11)	0.00±0.00	0.14±0.14	0.33±0.17	0.33±0.17	0.33±0.17
	SDZ Ser 082 (10)	0.00±0.00	0.08±0.05	0.25±0.11	0.45±0.19	0.45±0.19
	LY 278,584 (10)	0.00±0.00	0.08±0.08	0.16±0.08	0.16±0.08	0.16±0.08
	SDZ Ser 082+LY 278,584 (12)	0.02±0.02	0.09±0.09	0.17±0.09	0.17±0.09	0.17±0.09

The number of animals used in each group is indicated in parentheses in the second column.

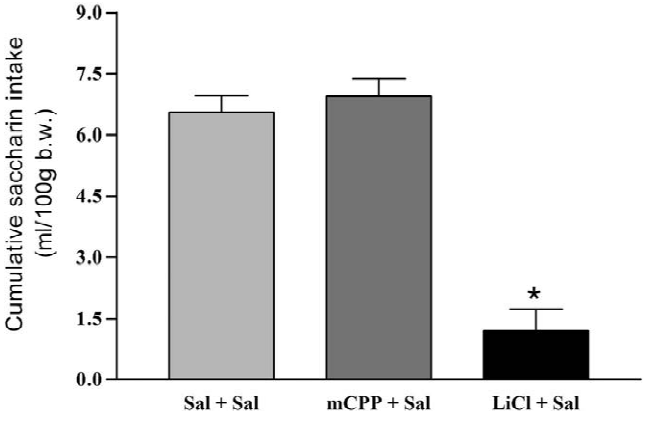

Fig. 5. Saccharin solution (0.25%) consumption (ml/100 g body weight) over 15 min at a second offering in animals receiving third ventricle injections of mCPP (240 nmol) or vehicle (controls). The sequence of injections used during the first saccharin offering and the number of animals used are indicated under each bar. The first injection was into the third ventricle and the second via intraperitoneal route. The asterisk indicates a statistically significant difference ($P<0.001$) between that particular group and controls (vehicle+vehicle).

that made an association between lithium chloride injections and saccharin intake showed a significantly lower intake of saccharin the following day, compared to controls. The association of mCPP injections at the highest dose employed in this study (240 nmol) with saccharin intake did not induce any significant decrease in saccharin intake in the following day. This seems to indicate that third ventricle injections of mCPP do not induce illness-like effects.

Table 2 shows urine output, renal sodium excretion and natremia 24 h after the first furosemide injection. Compared to saline-treated controls, furosemide significantly increased urine flow and renal sodium excretion. Plasma sodium concentration is significantly lower in furosemide-treated rats, when compared to controls.

Fig. 6 shows the effects of third ventricle injections of

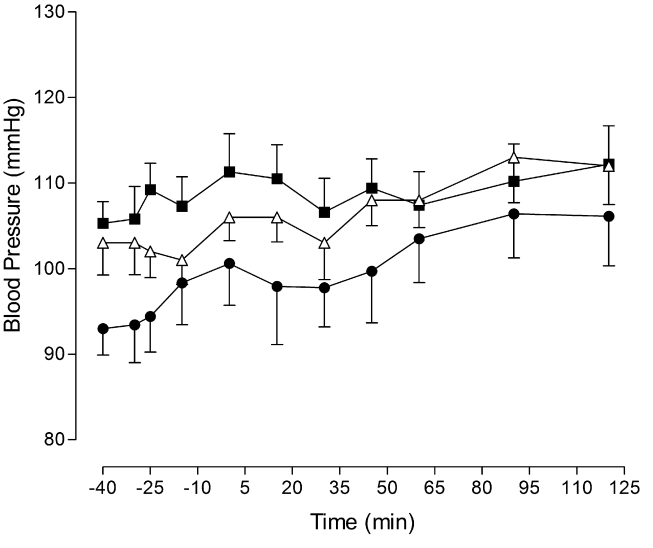

Fig. 6. Mean blood pressure (MBP) in sodium-depleted rats after third ventricle injections of vehicle (△; $n=8$), mCPP 240 nmol (●; $n=8$) or m-CPBG 240 nmol (■; $n=9$). Blood pressure recording between times −40 and −30 min corresponds to a pre-drug basal evaluation. Third ventricle injections were made at time −30 min. Data are presented as mean±S.E.M.

Table 2
Urine flow, renal sodium excretion and plasma sodium concentration in animals receiving s.c. injections of furosemide or vehicle

Treatment (*n*)	Urine flow (μl/min per 100 g)	Renal sodium excretion (nEquiv./min per 100 g)	Natremia (mEquiv./l)
Saline (12)	4.88±0.16	168.94±10.22	139.00±1.25
Furosemide (12)	9.84±0.38*	619.17±19.90*	129.20±0.64*

The number of animals used in each experiment is indicated in parentheses in column 1.
*$P<0.5$.

vehicle, mCPP or m-CPBG (240 nmol in each case) on blood pressure in rats submitted to sodium depletion using the same experimental protocol employed to study salt intake. Here, the central administration of both mCPP and m-CPBG was unable to modify blood pressure compared to controls.

4. Discussion

The present data clearly show that pharmacological stimulation of central 5-$HT_{2B/2C}$ and 5-HT_3 receptors significantly reduces salt intake in sodium-depleted rats. This reduction in salt intake, elicited by third ventricle injections of 5-$HT_{2B/2C}$ and 5-HT_3 agonists, does not seem to occur as a consequence of an acute increase in blood pressure.

Salt appetite and thirst are important behaviors which help mammals to regulate plasma osmolarity, blood volume and blood pressure, basic conditions that allow tissular perfusion. Specialized structures located both in the central nervous system and in strategic peripheral sites detect changes continuously and accurately in those parameters. Information obtained by these sensors feeds a complex central circuitry involving many brain areas and neurotransmitters, whose final corrective responses may be the stimulation or the inhibition of water and salt intake [20].

Structures surrounding the brain ventricles seem to be very important in salt intake regulation. Indeed, sodium depletion induces activation of areas such as the organum vasculosum laminae terminalis and the subfornical organ [31], and a significant reduction in sodium appetite in sodium-depleted animals is observed when those circumventricular structures are lesioned [10]. In addition, changes in sodium concentration in the cerebrospinal fluid are able to modify salt intake in rats after sodium depletion [9].

Brain serotonin pathways seem to exert important effects in the control of sodium appetite. The peripheral administration of dexfenfluramine, a serotonin uptake inhibitor and releaser, significantly reduces salt intake in sodium-depleted rats [26]. Furthermore, salt intake elicited by water deprivation is also inhibited by peripheral administration of MK-212, a 5-HT_{2C} agonist [13,25]. Also, mCPP, the same 5-$HT_{2B/2C}$ agonist that significantly inhibited salt intake when injected into the third ventricle in the present study, is able to reduce salt appetite when injected by a peripheral route [12]. Some conflicting results are also available in the literature showing that ketanserin, a 5-HT_2 receptor antagonist, may inhibit salt intake in two different experimental models: mineralocorticoid (DOCA) administration and sodium depletion [17]. However, ketanserin exhibits an important α1-adrenoceptor antagonistic activity [19], a fact that causes some uncertainty regarding how to interpret those data.

Insofar as we know, only a few studies have attempted to determine the specific location of brain serotonin circuitries and central serotonin receptor subtypes involved with water and salt intake regulation. Brainstem neurons placed in specific clusters within the midline raphe give origin to the central serotonin pathways that spread into many brain regions making serotonin a rather ubiquitous neurotransmitter [3]. Nevertheless, in rats, the blockade of serotonergic transmission in the lateral parabrachial nucleus significantly increases salt intake induced by the injection of angiotensin II into the subfornical organ [11]. Salt intake elicited by the combined administration of furosemide and captopril (a protocol that simultaneously induces sodium depletion and hypotension in rats) is also significantly increased by serotonin blockers injected into the lateral parabrachial nucleus [23]. These data seem to indicate that a major serotonergic pathway linking the area postrema/nucleus of solitary tract region to the lateral parabrachial nucleus exerts an important inhibitory drive on salt intake.

One focus of interest in this laboratory is the screening of the central receptors participating in water and salt intake regulation. The emerging picture shows a rather constant inhibitory effect on water intake observed after the stimulation of several brain serotonin receptors. Pharmacological stimulation of central 5-HT_{1D} receptors therefore inhibits water intake induced by a physiological drive (water deprivation) and after pharmacological stimulation of brain angiotensinergic and cholinergic pathways [14]. We have also demonstrated [8] that third ventricle injections of a 5-$HT_{2B/2C}$ agonist significantly reduce water intake elicited by distinct thirst-inducing physiological stimuli (water deprivation, hypovolemia and hyperosmolarity). More recently, we have shown that third ventricle injections of m-CPBG, a selective 5-HT_3 agonist, are also able to inhibit water intake evoked by central angiotensinergic and cholinergic pharmacological activation and after well-established dipsogenic physiological stimuli [6]. Generally, the same structures and neurotransmitters involved with the regulation of sodium appetite strongly influence water intake [20,15]. The present data, showing a significant inhibition of sodium appetite after central stimulation of 5-$HT_{2B/2C}$ and 5-HT_3 receptors, clearly indicate that brain serotonin uses the same receptor subtypes to achieve two different but well-correlated behaviors (water intake and sodium appetite) that deeply influence blood volume, blood pressure and plasma osmolarity.

Changes in blood pressure strongly influence sodium appetite [20]. Sodium intake is more promptly developed during hypovolemia if the animals are made simultaneously hypotensive [20]. Indeed, the role of blood pressure in the regulation of sodium appetite is clearly demonstrated by some studies showing that after the combined administration of furosemide plus captopril, a treatment that induces sodium depletion, salt intake is significantly

reduced if blood pressure is not allowed to decrease using sympathomimetic drugs such as phenylephrine [28]. On the other hand, acute increases in blood pressure may inhibit sodium appetite. Sodium-depleted sheep display a significant inhibition in salt intake when their blood pressure is maintained elevated [5]. Additionally, baroreceptor denervation significantly decreases sodium appetite in rats after sodium depletion [29]. In summary, hypotension seems to stimulate whereas hypertension inhibits salt intake.

In the present paper, the pharmacological stimulation of central 5-$HT_{2B/2C}$ and 5-HT_3 receptors was unable to modify blood pressure in rats submitted to the same experimental sodium depletion protocol used to study salt intake.

The drug mCPP is a 5-$HT_{2B/2C}$ receptor agonist whereas m-CPBG is considered to display specific agonistic properties toward 5-HT_3 receptors. Pretreatment with selective antagonists (SDZ SER 082 for 5-$HT_{2B/2C}$ and LY-278,584 for 5-HT_3 receptors) significantly blocks the reduction in salt intake generated by those agonists. Therefore, it is rational to conclude that the salt intake inhibition observed here in sodium-depleted rats is specifically due to the central blockade of 5-$HT_{2B/2C}$ and 5-HT_3 receptors. Additionally, when each one of those antagonists was injected alone into the third ventricle, no change in sodium appetite was observed. This strongly suggests the absence of an endogenous inhibitory drive on salt intake exerted by serotonin acting on 5-$HT_{2B/2C}$ and 5-HT_3 receptors, at least in sodium-depleted rats. No effect was seen when a combination of both antagonists was injected into the third ventricle. This reveals the absence of a salt intake-inducing drive exerted by an endogenous simultaneous activation of central 5-$HT_{2B/2C}$ and 5-HT_3 receptors. Some authors have found that brain serotonin may exert a tonic endogenous inhibition in salt intake [26]. However, they used metergoline as a pharmacological tool, a drug that may block several serotoninergic receptor subtypes other than those specifically studied in the present paper.

The pharmacological agent mCPP may display some affinity to 5-HT_{2A} receptors. It may therefore be coherent to propose an additional experimental protocol designed to test if 5-HT_{2A} receptor antagonists could modify the effects of mCPP observed here. However, prominent effects on non-serotonergic receptors or binding with less affinity to 5-HT_{2B} and 5-HT_{2C} receptors are important pharmacological properties of 5-HT_{2A} antagonists [18]. Hence, such a protocol would yield more doubts than certainties.

Inhibitory effects on ingestive behaviors in experimental protocols in animals may be consequent to actions on brain sites induced by the specific measures employed in that particular protocol. Alternatively, such inhibition may be the result of aversive effects associated with those procedures, whose strength is insufficient to produce visible signs of animal discomfort or distress but is able to interrupt non-adaptative behaviors such as feeding or drinking. Also, drugs affecting locomotor activity could mimic a true disruption in ingestive behavior by canceling or reducing the necessary motor events that allow the animal to approach the site where the product to be ingested is stored. We have recently demonstrated that m-CPBG does not produce any illness-like effect, as evidenced by a specific aversion test. The drug is also unable to inhibit the hedonic ingestion of a dessert meal and does not inhibit food intake [6]. We have also shown that mCPP does not disrupt hedonic ingestive behaviors since it does not modify the ingestion of saccharin offered as a dessert meal [8]. In the present paper, we have additionally shown using a classical aversion test that third ventricle mCPP injections are not associated with any aversive condition. Thus, it is logical to infer that the salt intake inhibition observed here is not consequent to a locomotor impairment or to a general sensation of discomfort or distress.

The brain serotonergic system is the target of a great number of legal and illegal drugs. The use of 5-HT_3 receptor antagonists as an antiemetic agent during radio and chemotherapy is rather common. In addition, several anxiolytic, antidepressant and antipsychotic drugs used in internal medicine and psychiatry interact with central 5-HT_2 receptors [22,2,33]. Thus, the present data explore new functional properties of a brain system that possesses significant clinical and therapeutical importance.

In summary, the present data clearly indicate that the pharmacological stimulation of central 5-$HT_{2B/2C}$ and 5-HT_3 receptors significantly reduces water intake in sodium-depleted rats. The experiments carried out here also demonstrate that central 5-$HT_{2B/2C}$ and 5-HT_3 receptors seem not to exert an endogenous inhibitory drive on salt intake in rats. In addition, it seems that the reduction in salt intake that follows central 5-$HT_{2B/2C}$ and 5-HT_3 pharmacological stimulation does not depend on an acute hypertensive response.

Acknowledgements

We are grateful to Vanilson Souza for his skilled technical assistance and to José de Souza for the animal care. The present work was supported through grants provided by the Brazilian Council of Research (CNPq), Process no. 46.0104/00-4 and by the Bahia State Research Support Financial Agency (CADCT).

References

[1] A. Abi-Dargham, M. Laruelle, D.T. Wong, D.W. Robertson, D.R. Weinberg, J.E. Kleinman, Pharmacological and regional characterization of [3H]LY278584 binding sites in human brain, J. Neurochem. 60 (1993) 730–737.

[2] S.A. Anttila, E.V. Leinonen, A review of the pharmacological and clinical profile of mirtazapine, CNS Drug Rev. 7 (2001) 249–264.
[3] N.M. Barnes, T. Sharp, A review of central 5-HT receptors and their function, Neuropharmacology 38 (1999) 1083–1152.
[4] P.H. Baylis, C.J. Thompson, Osmoregulation in health and disease, Clin. Endocrinol. 29 (1988) 549–576.
[5] E. Bott, D.A. Denton, S. Weller, The effect of angiotensin II infusion, renal hypertension and nephrectomy on salt appetite of sodium-deficient sheep, Aust. J. Exp. Biol. Med. Sci. 45 (1967) 595–612.
[6] L. Castro, B. Varjão, I. Maldonado, I. Campos, B. Duque, J. Fregoneze, I.R. De Oliveira, E. De-Castro-e-Silva, Central 5-HT_3 receptors and water intake in rats, Physiol. Behav. 77 (2002) 349–359.
[7] L. Castro, E. De Castro-e-Silva, C.P. Luz, A.K. Lima, F. Souza, I. Maldonado, D.F. Macêdo, M.G. Ferreira, G.F. Santamaria, I.P. Bandeira, A.L. Amor, F.L. Carvalho, M.A. Rocha Jr, J. Fregoneze, Central 5-HT_4 receptors and drinking behavior, Pharmacol. Biochem. Behav. 66 (2000) 443–448.
[8] L. Castro, I. Maldonado, I. Campos, B. Varjão, A.L. Angelo, R.A. Athanazio, M.C. Barbetta, A.C. Ramos, J. Fregoneze, E. De-Castro-e-Silva, Central administration of mCPP, a serotonin 5-$HT_{2B/2C}$ agonist, decreases water intake in rats, Pharmacol. Biochem. Behav. 72 (2002) 891–898.
[9] E. Chiaraviglio, M.F. Perez Guaita, The effect of intracerebroventricular hypertonic infusion on sodium appetite in rats after peritoneal dialysis, Physiol. Behav. 37 (1986) 695–699.
[10] E. Chiaraviglio, M. Perez Guaita, Anterior third ventricle (A3V) lesions and homeostasis regulation, J. Physiol. 79 (1984) 446–452.
[11] D.S.A. Colombari, J.V. Menani, A.K. Johnson, Forebrain angiotensin type 1 receptors and parabrachial serotonin in the control of NaCl and water intake, Am. J. Physiol. 271 (1996) 1470–1476.
[12] S.J. Cooper, R. Ciccocioppo, Effects of selective 5-HT_1 receptor agonist in water-deprived rats on salt intake in two-choice tests, Pharmacol. Biochem. Behav. 45 (1993) 513–518.
[13] S.J. Cooper, D.J. Barber, Effects of *d*-fenfluramine, MK-212, and ondansetron on saline drinking in two-choice tests in the rehydrating rat, Pharmacol. Biochem. Behav. 45 (1993) 593–596.
[14] E. De Castro-e-Silva, C. Sarmento, T.A. Nascimento, C.P. Luz, T. Soares, C.A. Marinho, M. Cunha, C. Bulcão, I.R. De Oliveira, J. Fregoneze, Effect of third ventricle administration of L-694,247, a selective 5-HT_{1D} receptor agonist, on water intake in rats, Pharmacol. Biochem. Behav. 57 (1997) 749–754.
[15] J.T. Fitzsimons, Angiotensin, thirst, and sodium appetite, Physiol. Rev. 78 (1998) 583–686.
[16] D.R. Gehlert, S.L. Gackenheimer, D.T. Wong, D.W. Robertson, Localization of 5-HT_3 receptor in the rat brain using [3H]LY278584, Brain Res. 553 (1991) 149–154.
[17] L. Gentili, A. Saija, G. Luchett, M. Massi, Effect of the 5-HT_2 antagonist ketanserin on salt appetite in the rat, Pharmacol. Biochem. Behav. 39 (1991) 171–176.
[18] D. Hoyer, J.P. Hannon, G.R. Martin, Molecular, pharmacological and functional diversity of 5-HT receptors, Pharmacol. Biochem. Behav. 71 (2002) 533–554.
[19] M. Israilova, F. Suzuki, T. Tanaka, T. Nagatomo, T. Taniguchi, I. Muramatsu, Binding and functional affinity of sarpogrelate, its metabolite m-1 and ketanserin for human recombinant alpha-1-adrenoceptor subtypes, Pharmacology 65 (2002) 69–73.
[20] A.K. Johnson, R.L. Thunhorst, The neuroendocrinology of thirst and salt appetite: visceral sensory signals and mechanisms of central integration, Front. Neuroendocrinol. 18 (1997) 292–353.
[21] R.S. Kahn, S. Wetzler, m-Chlorophenylpiperazine as a probe of serotonergic function, Biol. Psychiatry 30 (1991) 1139–1166.
[22] S. Kapur, R.B. Zipursky, G. Remington, Clinical and theoretical implications of 5-HT_2 and D2 receptor occupancy of clozapine, risperidone, and olanzapine in achizophrenia, Am. J. Psychiatry 156 (1999) 286–293.
[23] J.V. Menani, D.S.A. Colombari, T.G. Beltz, R.L. Thunhorst, A.K. Johnson, Salt appetite: interactions of forebrain angiotensinergic and hindbrain serotonergic mechanisms, Brain Res. 801 (1998) 29–35.
[24] J.V. Menani, R.L. Thunhorst, A.K. Johnson, Lateral parabrachial nucleus and serotonergic mechanisms in the control of salt appetite in rats, Am. J. Physiol. 270 (1996) 162–168.
[25] J.C. Neil, S.J. Cooper, Selective reduction by serotonergic agents of hypertonic saline consumption in rats: evidence for possible 5-HT_{1C} receptor mediation, Psychopharmacology 99 (1989) 196–201.
[26] M. Rouah-Rosilio, M. Orosco, S. Nicolaidis, Serotoninergic modulation of sodium appetite in the rat, Physiol. Behav. 55 (1994) 811–816.
[27] M.I. Sepúlveda, S.C. Lummis, I.L. Martin, The agonist properties of m-chlorophenylbiguanide and 2-methyl-5-hydroxytryptamine on 5-HT_3 receptors in N1E-115 neuroblastoma cells, Br. J. Pharmacol. 104 (1991) 536–540.
[28] R.L. Thunhorst, A.K. Johnson, Renin-angiotensin, arterial blood pressure and salt appetite in rats, Am. J. Physiol. 266 (1994) R458–R465.
[29] R.L. Thunhorst, S.J. Lewis, A.K. Johnson, Effects of sinoaortic baroreceptor denervation on depletion-induced salt appetite, Am. J. Physiol. 267 (1994) R1043–R1049.
[30] J.A. Van Hooft, H.P. Vijverberg, Full and partial agonists induce distinct desensitized states of the 5-HT_3 receptor, J. Recept. Signal Transduct. Res. 17 (1997) 267–277.
[31] L. Vivas, C.V. Pastuskovas, L. Tonelli, Sodium depletion induces Fos immunoreactivity in circumventricular organs of the lamina terminalis, Brain Res. 679 (1995) 34–41.
[32] D.L. Willins, H.Y. Meltzer, Direct injection of 5-HT_{2A} receptor agonist into the medial prefrontal cortex produces a head-twitch response in rats, J. Pharmacol. Exp. Ther. 282 (1997) 699–706.
[33] M.D. Wood, C. Reavill, B. Trail, A. Wilson, T. Stean, G.A. Kennett, S. Lightowler, T.P. Blackburn, D. Thomas, T.L. Gager, G. Riley, V. Holland, S.M. Bromidge, I.T. Forbes, D.N. Middlemiss, SB-243213; a selective 5-HT_{2C} receptor inverse agonist with anxiety, Neuropharmacology 41 (2001) 186–199.