available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

The central amygdala regulates sodium intake in sodium-depleted rats: Role of 5-HT$_3$ and 5-HT$_{2C}$ receptors

C.P. Luz[a], A. Souza[b], R. Reis[b], P. Mineiro[b], H.S. Ferreira[c], J.B. Fregoneze[b], E. De Castro e Silva[b,*]

[a]Department of Biological Sciences, State University of Southwest Bahia 45200-000, Jequié, Bahia, Brazil
[b]Department of Physiology, Health Sciences Institute, Federal University of Bahia, 40110-100 Salvador, Bahia, Brazil
[c]Life Sciences Department, Bahia State University, 41195-001 Salvador, Bahia, Brazil

ARTICLE INFO

Article history:
Accepted 3 January 2007
Available online 9 January 2007

Keywords:
Serotonin
5HT2 receptor
5HT3 receptor
Salt intake
Water intake
Thirst
Amygdala
Central amygdala

ABSTRACT

In the present paper, we have evaluated the participation of 5-HT$_3$ and 5-HT$_{2C}$ receptors in the central amygdala (CeA) in the regulation of water and salt intake in sodium-depleted rats. m-CPBG-induced pharmacological activation of 5-HT$_3$ receptors located in the CeA resulted in a significant reduction in salt intake in sodium-depleted rats. This antinatriorexic effect of m-CPBG was reverted by pretreatment with the selective 5-HT$_3$ receptor antagonist ondansetron. The injection of ondansetron alone into the CeA had no effect on sodium-depleted and normonatremic rats. Conversely, pharmacological stimulation of 5-HT$_{2C}$ receptors located in the central amygdala by the selective 5-HT$_{2C}$ receptor agonist m-CPP failed to modify salt intake in sodium-depleted rats. Additionally, the administration of a selective 5-HT$_{2C}$ receptor blocker, SDZ SER 082, failed to modify salt intake in rats submitted to sodium depletion. These results lead to the conclusion that the pharmacological activation of 5-HT$_3$ receptors located within the CeA inhibits salt intake in sodium-depleted rats and that 5-HT$_{2C}$ receptors located within the CeA appear to be dissociated from the salt intake control mechanisms operating in the central amygdala.

1. Introduction

In a series of previous studies, we have tried to establish the role of several brain serotonergic receptors in the control of water and salt intake. Our results showed that the activation of central 5-HT$_{1D}$ receptors by selective pharmacological agents leads to a significant inhibition of water intake induced by central cholinergic, angiotensinergic and adrenergic stimulation (De Castro e Silva et al., 1997). Furthermore, we have demonstrated that central 5-HT$_4$ receptors seem to exert a dualistic role in the control of water intake, potentiating angiotensin II-induced drinking and inhibiting thirst induced by central cholinergic activation (Castro et al., 2000). We have also shown that the 5-HT$_2$ receptor family appears to participate in thirst and sodium appetite regulation since the pharmacological activation of central 5-HT$_{2C}$ receptors inhibits water intake elicited by different thirst-inducing physiological stimuli (Castro et al., 2002a) and decreases sodium appetite in sodium-depleted rats (Castro et al., 2003). In addition, we have established that the central activation of 5-HT$_3$ receptors significantly decreases water intake in experimental protocols in which different physiological

* *Corresponding author*. Departamento de Biorregulação, Instituto de Ciências da Saúde, Universidade Federal da Bahia, 40110-100 Salvador-BA, Brazil.
E-mail address: emilio@ufba.br (E. De Castro e Silva).

0006-8993/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2007.01.012

stimuli are used to promote thirst (dehydration, hypovolemia and hyperosmolarity) and following central angiotensinergic and cholinergic activation (Castro et al., 2002b). We have also shown that the pharmacological stimulation of central 5-HT_3 receptors reduces salt intake in sodium-depleted rats (Castro et al., 2003). In all of the above-mentioned studies, the pharmacological manipulations resulted from intracerebroventricular injections of selective serotonergic agents.

Multiple serotonin receptors exert a myriad of physiological effects depending on the regions in which they are situated within the central nervous system (Uphouse, 1997). Therefore, the investigation of the roles played by the several

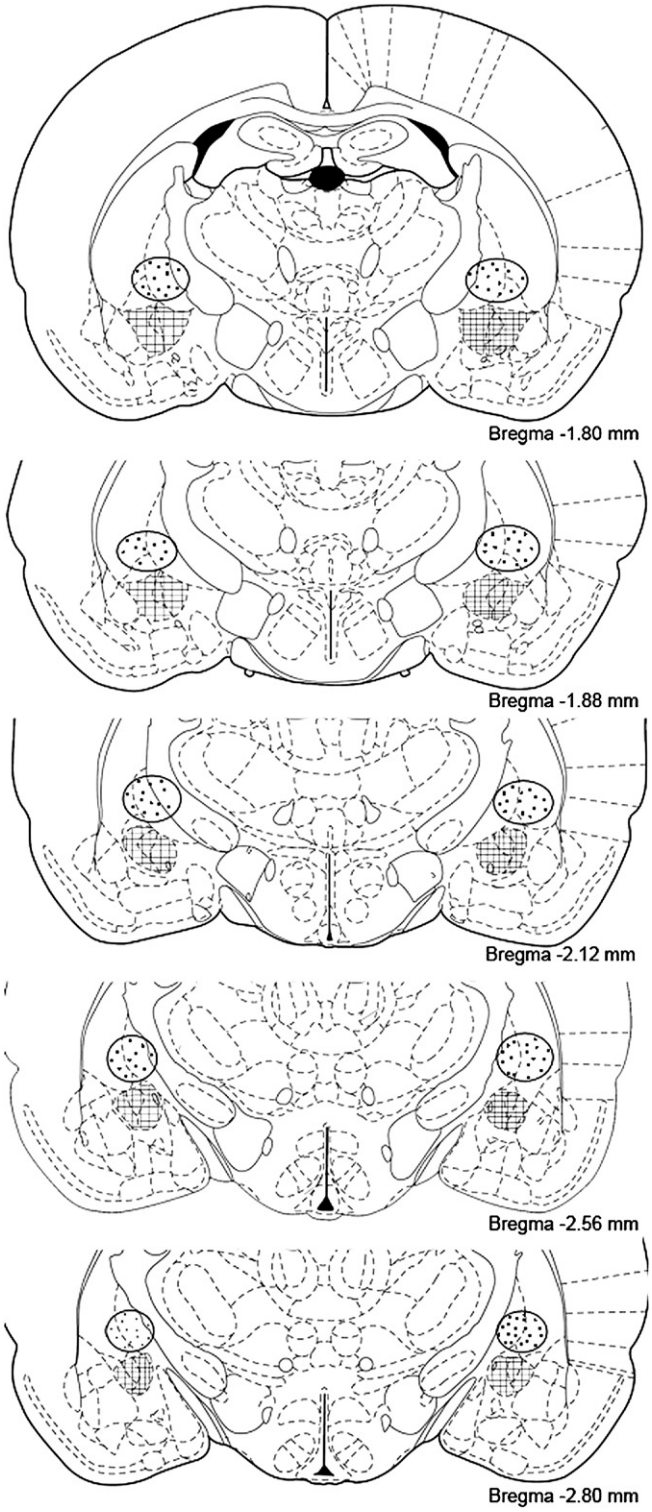

Fig. 1 – Diagram based on the Paxinos and Watson Atlas showing sequential coronal sections of areas reached by the injections within (hachured area) and outside (dotted area) the central amygdala.

serotonergic receptors located in specific brain regions on the regulation of water and salt intake is a logical next step in our research path.

The amygdala, a brain region containing distinct nuclei, influences a great number of behaviors tailored to promote the animal's adaptation to external and internal stimuli (Price et

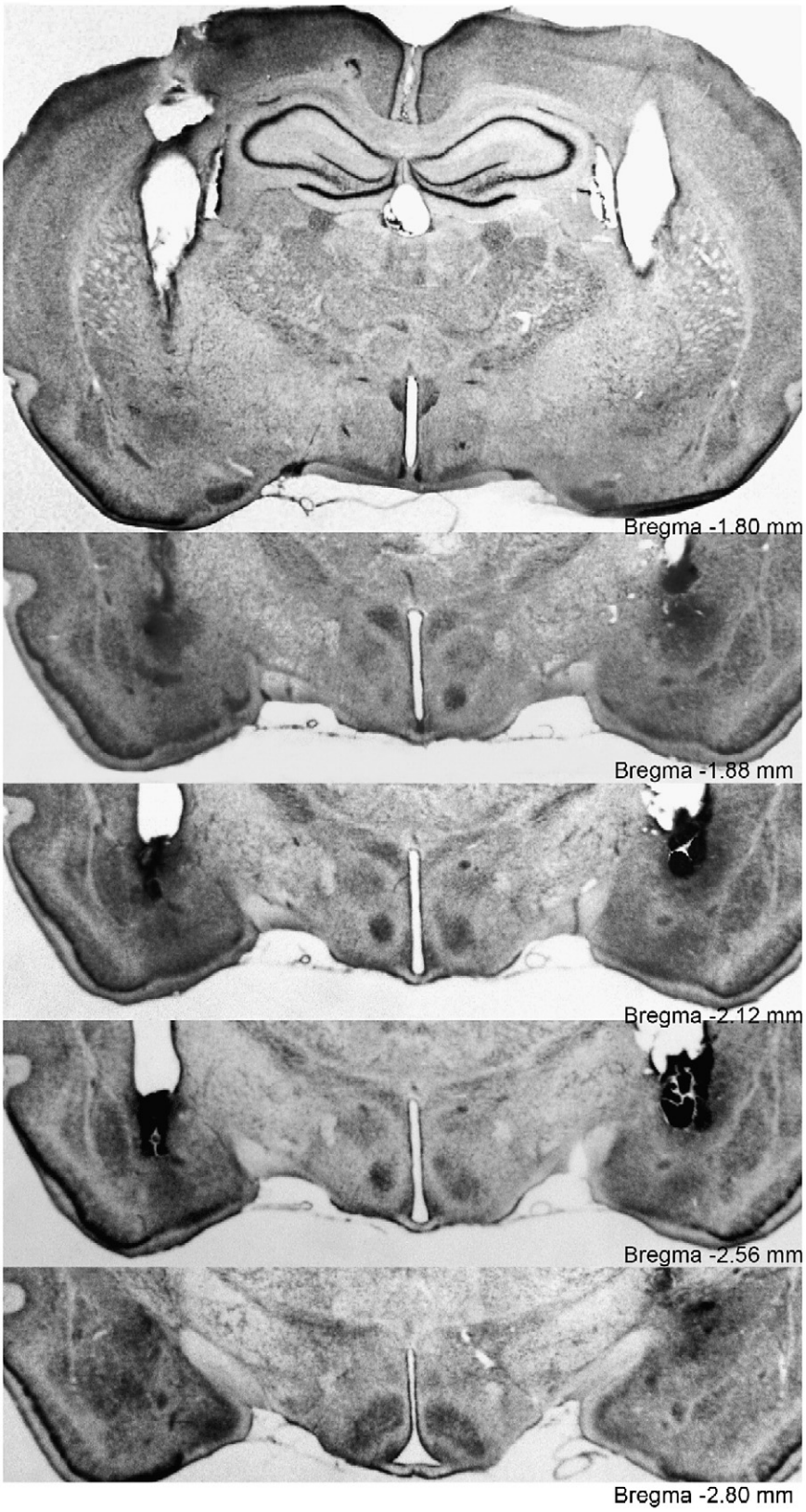

Fig. 2 – Photomicrographs showing the sequential coronal sections of the areas reached by typical bilateral injections into the central amygdala.

al., 1987), and seems to participate in the regulation of salt intake (Johnson et al., 1999). The distinct nuclei within the amygdala may play particular roles in the control of water and salt intake. Indeed, it has been clearly demonstrated that the basolateral, central and medial nuclei of the amygdala seem to influence sodium appetite in several experimental circumstances. Surgical lesions of the basolateral amygdala inhibit salt intake induced by mineralocorticoid treatment (Nachman and Ashe, 1974). Also, lesions of the central amygdala (CeA) reduce spontaneous sodium intake, as well as sodium appetite induced by pharmacological procedures such as subcutaneous injections of yohimbine and central angiotensinergic stimulation (Woodwar et al., 1979; Galaverna et al., 1992). The role of the several nuclei of the amygdala in the control of sodium appetite seems to be linked to the physiological conditions triggering the intake of sodium since lesions of the medial amygdala (MeA) impair mineralocorticoid-induced salt intake but do not affect salt intake promoted by sodium depletion (Nitabach et al., 1989; Zhang et al., 1993).

We have recently demonstrated that both 5-HT_3 and 5-HT_{2C} receptors in the amygdala influence sodium appetite since the pharmacological activation of 5-HT_3 receptors located within the MeA inhibits salt intake and, in this brain region, the functional integrity of 5-HT_{2C} receptors is required for the full expression of sodium appetite when rats are sodium-depleted (Luz et al., 2006).

Since the different nuclei in the amygdala may play distinct roles in the control of salt intake and since multiple serotonin receptors may exhibit distinct physiological roles depending

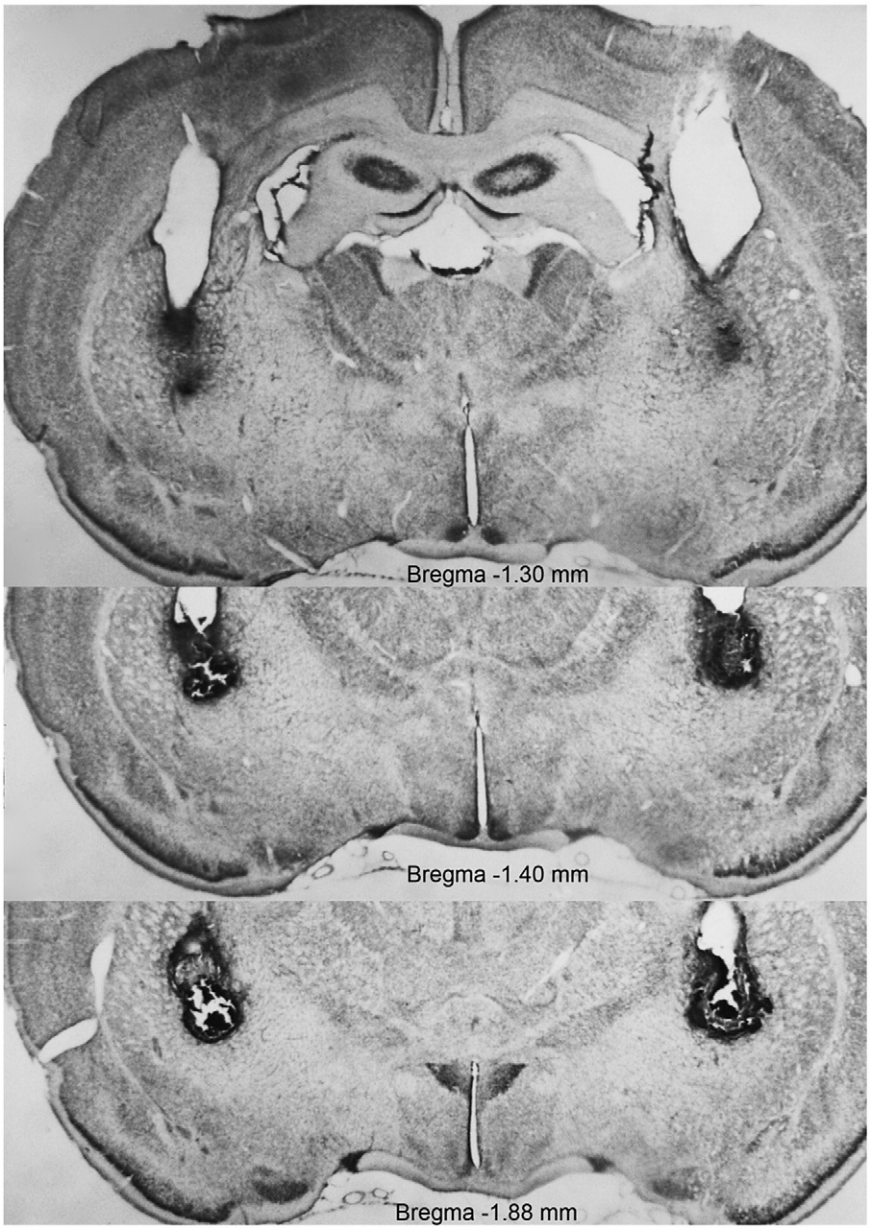

Fig. 3 – Photomicrographs showing the sequential coronal sections of the areas reached by the injections outside the central amygdala.

on their location within the central nervous system, in the present study, we decided to investigate the role of 5-HT_3 and 5-HT_{2C} receptors within the CeA on water and salt intake in rats submitted to sodium depletion.

2. Results

Fig. 1 corresponds to a diagram based on the Paxinos and Watson Atlas showing sequential coronal sections of the areas reached by injections within and outside the central amygdala. Fig. 2 corresponds to photomicrographs showing the sequential coronal sections of the areas reached by typical bilateral injections into the central amygdala. Fig. 3 corresponds to photomicrographs showing the sequential coronal sections of the areas reached by the injections outside the central amygdala.

Table 1 displays the water and salt intake obtained with animals that received misplaced injections of m-CPBG and ondansetron. No effect was observable when these drugs were injected into sites located outside CeA.

Fig. 4A shows the effect of bilateral injections of m-CPBG, a selective 5-HT_3 receptor agonist, on salt intake in rats rendered sodium-depleted by previous subcutaneous furosemide injections. At the lowest dose used (10 nmol), the injection of the drug caused no effect. At all other doses used (40, 80, 160 nmol), m-CPBG induced a significant decrease in salt intake as compared to control, sodium-depleted rats receiving bilateral injections of isotonic saline solution into the CeA. The magnitude of salt intake inhibition seems to be proportional to the dose used. Indeed, after 120 min, rats receiving m-CPBG at the dose of 40 nmol have a total salt intake approximately one-third of that presented by the control, saline-treated group, while the salt intake of sodium-depleted animals receiving m-CPBG at the highest dose of 160 nmol is totally inhibited. As expected, control, normonatremic rats (those receiving subcutaneous injections of isotonic saline solution and treated with bilateral saline injections into the CeA) present negligible salt intake. Panel B shows, as expected, that there was no water intake in any of the groups studied (sodium-depleted and control, normonatremic animals).

Fig. 5A shows the effect of the blockade of 5-HT_3 receptors by the selective antagonist ondansetron on the antinatriorexic effect of m-CPBG. Here, as expected, a high salt intake was recorded in control, sodium-depleted rats receiving injections of isotonic saline solution into the CeA. Bilateral injections of m-CPBG into the CeA (160 nmol) significantly blocked salt intake in sodium-depleted rats, and the pretreatment of this group of rats with ondansetron extinguished the antinatriorexic effect of m-CPBG. Similar to the events depicted in Fig. 4A, no salt intake was recorded in control, normonatremic rats (those receiving subcutaneous injections of isotonic saline solution and treated with bilateral saline injections into the CeA) which present no salt intake. Panel B shows, as expected, that there was negligible

Table 1 – Misplaced injections of m-CPBG, ondansetron or saline outside the central amygdala of sodium-depleted animals

Treatment		Time					
	Intakes	15	30	45	60	90	120
Saline (8)	Water	0.08±0.04	0.09±0.04	0.16±0.06	0.16±0.06	0.20±0.10	0.20±0.10
	Salt	1.16±0.38	2.60±0.50	3.97±0.52	4.22±0.60	4.94±0.69	5.15±0.65
m-CPBG 160 nmol (9)	Water	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Salt	1.79±0.51	3.15±0.70	3.70±0.75	3.91±0.64	4.57±0.32	4.71±0.33
m-CPBG 80 nmol (6)	Water	0.35±0.35	0.35±0.35	0.44±0.38	0.50±0.38	0.50±0.38	0.52±0.38
	Salt	1.03±0.52	1.74±0.71	2.40±0.84	2.83±0.89	4.43±0.30	5.02±0.39
m-CPBG 40 nmol (8)	Water	0.04±0.04	0.07±0.07	0.07±0.07	0.07±0.07	0.07±0.07	0.07±0.07
	Salt	1.25±0.37	2.42±0.49	2.94±0.54	3.71±0.53	4.50±0.58	5.25±0.39
m-CPBG 10 nmol (5)	Water	0.00±0.00	0.02±0.02	0.02±0.02	0.02±0.02	0.02±0.02	0.02±0.02
	Salt	1.86±0.30	2.50±0.29	3.10±0.38	3.18±0.40	3.99±0.50	4.34±0.54
ANOVA	Water	$F_{(4,31)}=1.07$; $p=0.39$	$F_{(4,31)}=0.97$; $p=0.44$	$F_{(4,31)}=1.32$; $p=0.28$	$F_{(4,31)}=1.65$; $p=0.19$	$F_{(4,31)}=1.58$; $p=0.20$	$F_{(4,31)}=1.78$; $p=0.16$
	Salt	$F_{(4,31)}=0.69$ $p=0.59$	$F_{(4,31)}=0.72$ $p=0.58$	$F_{(4,31)}=0.90$ $p=0.48$	$F_{(4,31)}=0.70$ $p=0.60$	$F_{(4,31)}=0.37$ $p=0.83$	$F_{(4,31)}=0.52$ $p=0.72$
Saline (5)	Water	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Salt	1.62±0.53	2.88±0.51	3.32±0.50	3.46±0.54	3.72±0.56	4.36±0.30
Ond 160 nmol (7)	Water	0.00±0.00	0.00±0.00	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01
	Salt	2.07±0.60	2.69±0.74	3.26±0.70	4.32±0.66	5.26±0.49	5.63±0.46
Ond 80 nmol (9)	Water	0.16±0.16	0.16±0.16	0.16±0.16	0.16±0.16	0.23±0.23	0.23±0.23
	Salt	1.41±0.48	2.44±0.58	3.53±0.34	4.16±0.28	4.61±0.23	4.99±0.23
Ond 40 nmol (10)	Water	0.02±0.02	0.02±0.02	0.05±0.04	0.05±0.04	0.05±0.04	0.05±0.04
	Salt	1.68±0.36	3.31±0.47	3.96±0.38	4.31±0.27	4.79±0.23	5.14±0.13
ANOVA	Water	$F_{(3,27)}=0.72$; $p=0.55$	$F_{(3,27)}=0.69$; $p=0.57$	$F_{(3,27)}=0.56$; $p=0.65$	$F_{(3,27)}=0.56$; $p=0.65$	$F_{(3,27)}=0.62$; $p=0.61$	$F_{(3,27)}=0.62$; $p=0.61$
	Salt	$F_{(3,27)}=0.32$; $p=0.81$	$F_{(3,27)}=0.47$; $p=0.71$	$F_{(3,27)}=0.48$; $p=0.70$	$F_{(3,27)}=0.70$; $p=0.56$	$F_{(3,27)}=2.60$; $p=0.07$	$F_{(3,27)}=2.74$; $p=0.06$

Results are shown as mean±SEM. There were no statistically significant differences among the groups. The number of animals used in each experimental set is indicated in parenthesis.

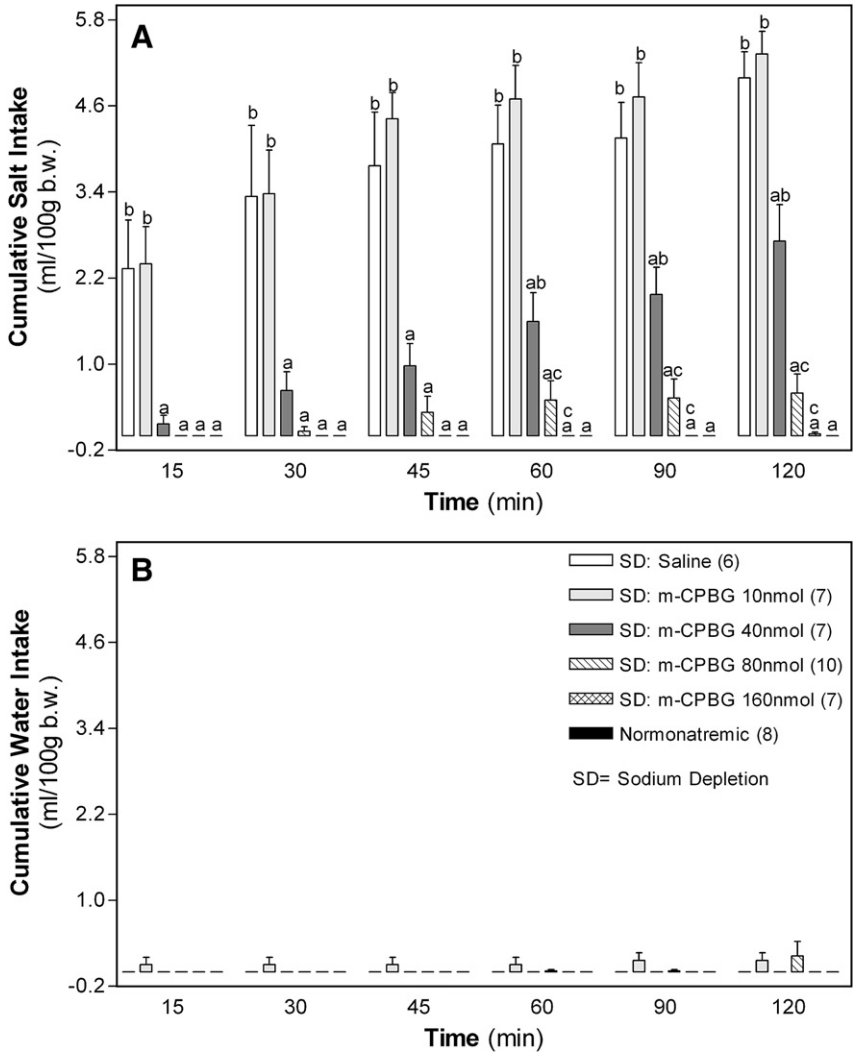

Fig. 4 – Cumulative salt (A) and water (B) intakes (ml/100 g body weight) of sodium-depleted animals treated with bilateral injections of m-CPBG, at various doses, into the CeA. The distinct pharmacological treatments and the number of animals used in each group are indicated within panel B. The animals labeled as normonatremic are control animals receiving subcutaneous injections of saline solution, instead of furosemide, and bilateral injections of isotonic saline solution into the CeA. Data are presented as mean ± SEM. "a" indicates a statistically significant difference (one-way ANOVA followed by Newman–Keul's test; $p<0.05$) when the distinct groups of animals are compared to control sodium-depleted animals receiving injections of saline into the CeA. "b" indicates a statistically significant difference when the group of rats not submitted to sodium-depletion is compared to sodium-depleted animals receiving saline and m-CPBG at various doses. "c" indicates a statistically significant difference ($p<0.05$) when sodium-depleted animals receiving injections of m-CPBG at the doses of 160 and 80 nmol into the CeA are compared to animals receiving m-CPBG at the dose of 40 nmol. Each bar in the graph has been obtained from a naive group of animals.

water intake in all groups studied (sodium-depleted and control, normonatremic animals).

Fig. 6A shows the effect of bilateral injections of ondansetron alone into the CeA on salt intake in sodium-depleted rats. Control, sodium-depleted rats receiving bilateral injections of isotonic saline solution presented a high salt intake as compared to control, normonatremic rats (those receiving subcutaneous injections of isotonic saline solution and treated with bilateral saline injections into the CeA). At all doses used (40, 80 and 160 nmol), bilateral injections of ondansetron were unable to modify the high salt intake presented by sodium-depleted animals. Panel B shows, as expected, that there was insignificant water intake in all groups studied (sodium-depleted and control, normonatremic animals).

Fig. 7 shows a comparison between the effect of m-CPBG in several doses when injected into the CeA (produced in the present paper) with the effect of the injection of m-CPBG at the same doses when injected into the MeA, as we have presented in a previous paper (Luz et al., 2006). It is clear that, at the same doses, m-CPBG elicits a more powerful inhibition of salt intake in sodium-depleted rats when injected into the CeA as compared to the effect produced by the same drug when injected into the MeA.

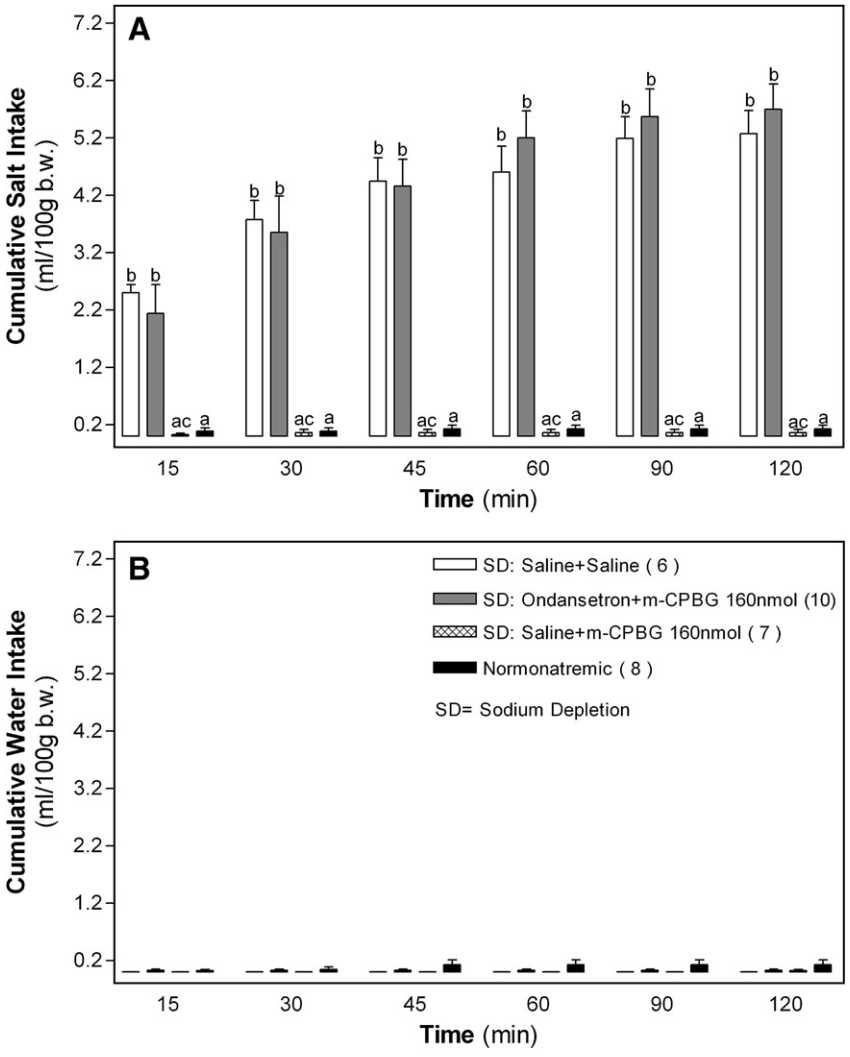

Fig. 5 – Cumulative salt (A) and water (B) intakes (ml/100 g body weight) of sodium-depleted animals treated with bilateral injections of m-CPBG (160 nmol) or saline into the CeA but pretreated with injections of ondansetron (160 nmol) or saline into the CeA. The distinct pharmacological treatments and the number of animals used in each group are indicated within panel B. The animals labeled as normonatremic are control animals receiving subcutaneous injections of saline solution, instead of furosemide, and bilateral injections of isotonic saline solution into the CeA. Data are presented as mean ± SEM. "a" indicates a statistically significant difference (one-way ANOVA followed by Newman–Keul's test; $p<0.05$) when the distinct groups of animals are compared to control sodium-depleted animals (saline + saline). "b" indicates a statistically significant difference when the group of rats not submitted to sodium-depletion is compared to the groups of sodium-depleted rats. "c" indicates a statistically significant difference when the group of sodium-depleted rats receiving saline + m-CPBG (160 nmol) into the CeA is compared to the group of sodium-depleted animals receiving ondansetron (160 nmol) + m-CPBG (160 nmol). Each bar in the graph has been obtained from a naive group of animals.

Fig. 8A shows the effect of bilateral injections of m-CPP, a selective 5-HT_{2C} receptor agonist, on salt intake in rats rendered sodium-depleted by previous subcutaneous furosemide injections. Salt intake in the groups of sodium-depleted rats receiving m-CPP, at any of the doses used (40, 80 and 160 nmol), is indistinguishable from that of the control, sodium-depleted rats receiving bilateral injections of isotonic saline solution into the CeA. As expected, there was negligible salt intake in the control, normonatremic rats (those receiving subcutaneous injections of isotonic saline solution and treated with bilateral saline injections into the CeA). Panel B shows, as expected, that there was no water intake in any of the groups studied (sodium-depleted and control, normonatremic animals).

Fig. 9A shows the effect of bilateral injections of SDZ SER 082 (a selective 5-HT_{2C} receptor antagonist) alone into the CeA on salt intake in sodium-depleted rats. A high salt-intake was registered in control, sodium-depleted rats receiving bilateral injections of isotonic saline solution as compared to control, normonatremic rats (those receiving subcutaneous injections of isotonic saline solution and treated with bilateral saline injections into the CeA). Bilateral injections of ondansetron were unable to modify the high salt intake of sodium-depleted animals at any of the doses used (40, 80 and 160 nmol). Panel B

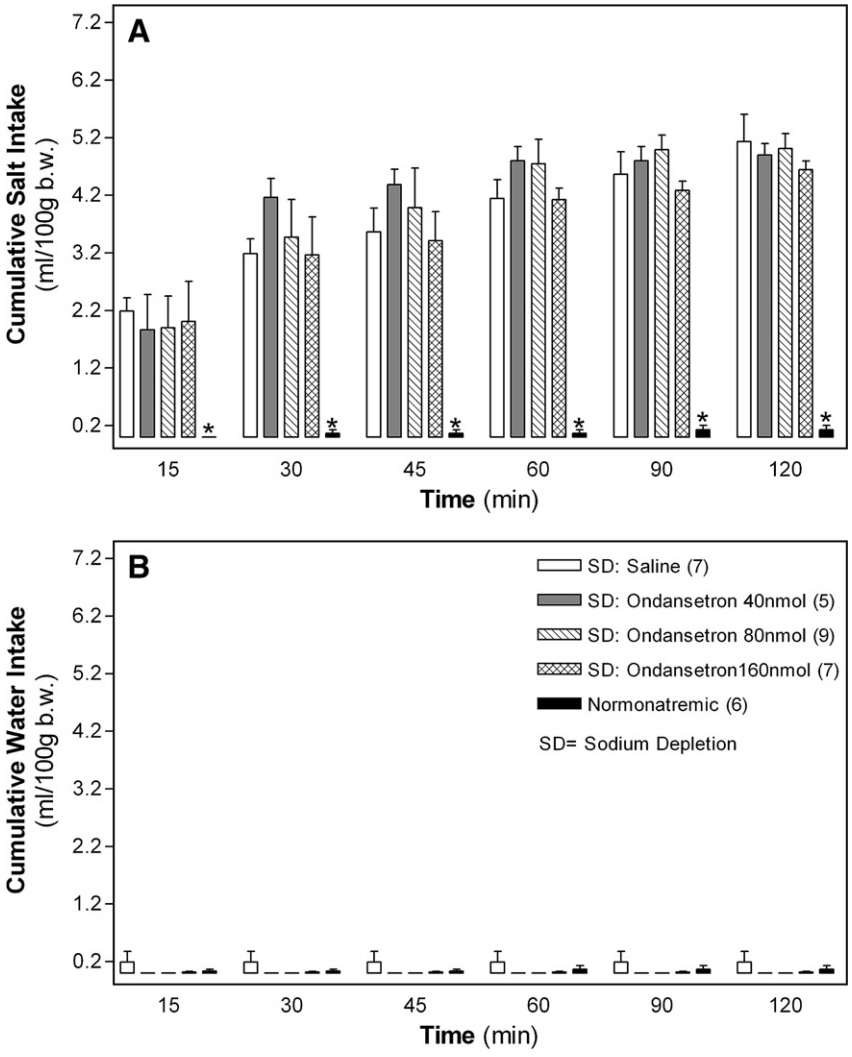

Fig. 6 – Cumulative salt (A) and water (B) intakes (ml/100 g body weight) of sodium-depleted animals treated with bilateral injections of ondansetron, at various doses, into the CeA. The distinct pharmacological treatments and the number of animals used in each group are indicated within panel B. The animals labeled as normonatremic are control animals receiving subcutaneous injections of saline solution, instead of furosemide, and bilateral injections of isotonic saline solution into the CeA. Data are presented as mean ± SEM. Asterisks indicate a statistically significant difference (one-way ANOVA followed by Newman–Keul's test; $p<0.05$) when the group of animals not submitted to sodium-depletion is compared to all other groups. Each bar in the graph has been obtained from a naive group of animals.

shows, as expected, the insignificant water intake registered in all the groups studied (sodium-depleted and control, normonatremic animals).

Table 2 shows the effect of bilateral injections of ondansetron (160 nmol) into the amygdala on water and salt intake in normonatremic animals. The administration of this compound did not induce any modification in those parameters compared to saline-treated controls.

Fig. 10A shows the results of the avoidance test performed to check whether the antinatriorexic effects of m-CPBG could be consequent to any "illness-like" side effects. Analysis of variance indicated a significant treatment difference between the groups [$F_{(2,15)}=93.3$; $p<0.0001$]. As expected, there was a significant reduction in saccharin intake on the following day in animals establishing a previous association between lithium chloride and saccharin as compared to saline-treated controls. Conversely, the previous association of m-CPBG with saccharin failed to produce any significant reduction in saccharin intake the next day, suggesting that it is improbable that illness-like effects could explain the results observed here after the injection of this compound into the central amygdala. Fig. 9B depicts the results of the dessert test. Here, saccharin intake was similar in saline-treated control animals and in animals receiving bilateral injections of m-CPBG into the CeA (160 nmol), indicating that the preferential intake of a "tasty" solution (a hedonic behavior) was not modified by central injections of m-CPBG ($t=1.79$; $df=10.0$; $p=0.10$).

Table 3 lists the values of F, df and p for the effects obtained after injection of the serotonergic agents used, alone or in combination, into the CeA, in the experimental sets designed to study the role of brain 5-HT_3 and 5-HT_{2C} receptors on the water and salt intake of sodium-depleted rats.

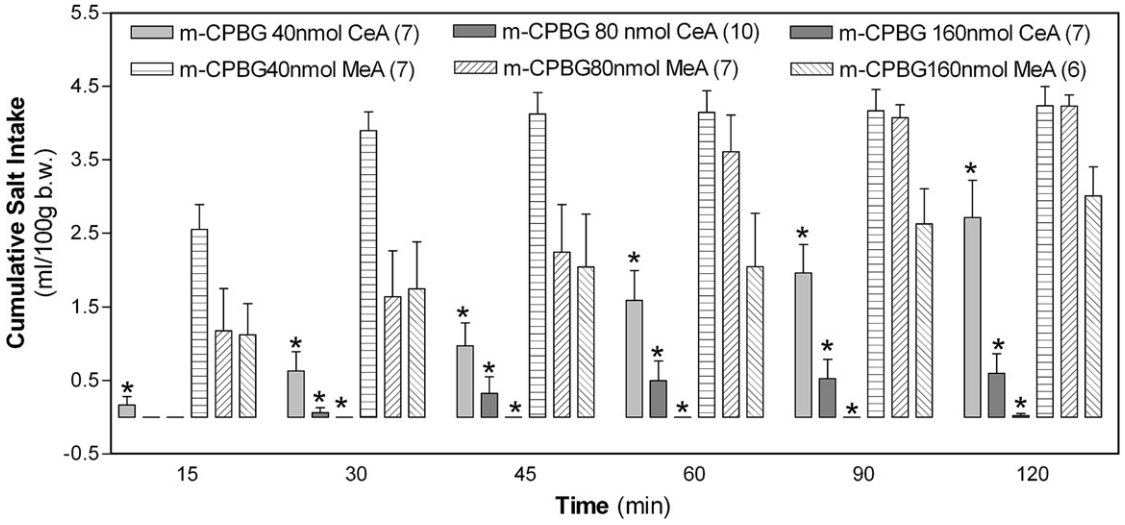

Fig. 7 – Cumulative salt intake (ml/100 g body weight) of sodium-depleted animals treated with bilateral injections of m-CPBG, at various doses, into the CeA and MeA. The distinct pharmacological treatments and the number of animals used in each group are indicated in the figure. Data are presented as mean±SEM. *Indicates a statistically significant difference (one-way ANOVA followed by Newman–Keul's test; $p<0.05$) when the distinct groups of animals receiving injections of m-CPBG into the CeA are compared to animals receiving injections of m-CPBG at the same doses into the MeA. The data on m-CPBG effects on salt intake when injected into MeA were produced in a previous paper (Luz et al., 2006).

Fig. 11 shows the effects of injections of m-CPBG (160 nmol) or saline solution into the CeA on blood pressure in rats submitted to sodium depletion using the same experimental protocol employed to study salt intake. In this situation, the central administration of m-CPBG was unable to modify blood pressure as compared to controls.

3. Discussion

The data shown in the present study demonstrate that the pharmacological activation of 5-HT_3 receptors located within the CeA, by the selective 5-HT_3 receptor agonist m-CPBG, significantly reduces salt intake in sodium-depleted rats. The antinatriorexic effect observed in this group of animals after the administration of m-CPBG seems to be due to the interaction of this compound with 5-HT_3 receptors since pretreatment with a selective 5-HT_3 antagonist, ondansetron, impairs the decrease in salt intake induced by the administration of this serotonergic agonist. It was also shown that bilateral injections of ondansetron alone into the CeA failed to modify salt intake in sodium-depleted and normonatremic rats. On the other hand, bilateral injections of m-CPP, a 5-HT_{2C} receptor agonist, and SDZ SER 082, a selective 5-HT_{2C} receptor antagonist, did not modify salt intake in sodium-depleted animals. The inhibitory action of m-CPBG on salt intake is not consequent to sickness-like effects elicited by its injection into the CeA since an aversion test ruled out this possibility. Also, the antinatriorexic effect obtained after bilateral injections of m-CPBG into the CeA is not due to a general, non-specific inhibitory effect on all ingestive behaviors, or to a locomotor deficit, since this pharmacological treatment failed to modify the ingestion of a palatable saccharin solution.

We have been investigating the role of central serotonergic receptors in the control of water and salt intake, as previously mentioned (Castro et al., 2000, 2002a,b, 2003; De Castro e Silva et al., 1997). In all these studies, we used a pharmacological approach based on third ventricle injections of selective serotonergic agents, a method that does not allow the identification of the brain regions in which a particular neurotransmitter acts to promote a specific effect. Recently, we decided to investigate the role of serotonergic receptors located in specific brain regions in the control of ingestive behaviors linked to hydrosaline homeostasis, by exploring the participation of 5-HT_3 and 5-HT_{2C} receptors located in discrete nuclei of the amygdala in the modulation of water and salt intake in sodium-depleted rats.

The amygdala, a brain structure that is connected to both prosencephalic and rhomboencephalic structures involved in the regulation of body sodium levels and sodium appetite, plays a well-documented role in the homeostasis of sodium (Johnson et al., 1999). A series of studies based on lesions of several nuclei of the amygdala has proven that this brain region influences sodium appetite in a way that makes this ingestive behavior a major regulatory loop in the control of sodium balance, together with renal sodium excretion/reabsorption and water intake. Indeed, surgical lesions of the MeA and CeA inhibit salt intake induced by the administration of mineralocorticoids (Nitabach et al., 1989; Schulkin et al., 1989; Galaverna et al., 1992; Zardetto-Smith et al., 1994).

The amygdaloid complex comprises more than 10 nuclei located in the midtemporal lobe that can be distinguished by cytoarchitectonic and connectional basis. Among these subparts, the centromedial nuclei consist of the central amygdala, the medial amygdala and the bed nucleus of the stria terminalis. The CeA is located dorsomedially in the rostral part of the amygdala having the basolateral complex as lateral

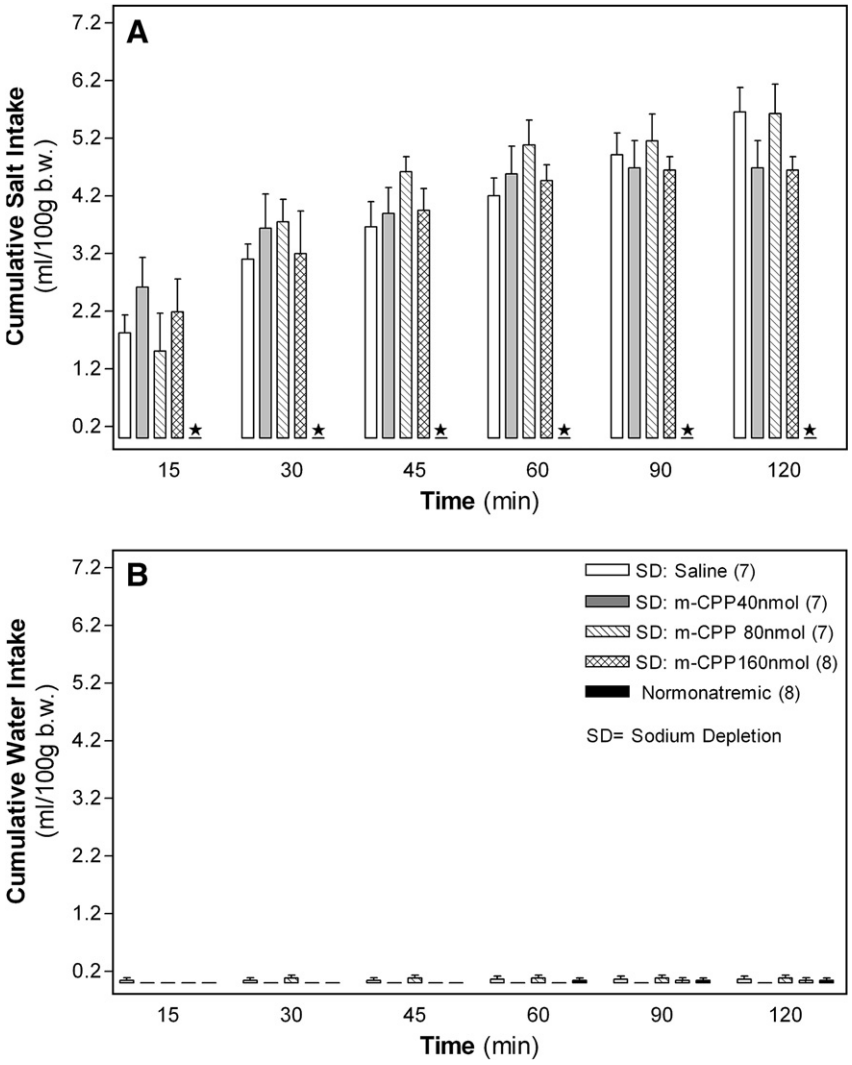

Fig. 8 – Cumulative salt (A) and water (B) intakes (ml/100 g body weight) of sodium-depleted animals treated with bilateral injections of m-CPP, at various doses, into the CeA. The distinct pharmacological treatments and the number of animals used in each group are indicated within panel B. The animals labeled as normonatremic are control animals receiving subcutaneous injections of saline solution, instead of furosemide, and bilateral injections of isotonic saline solution into the CeA. Data are presented as mean ± SEM. Asterisks indicate a statistically significant difference (one-way ANOVA followed by Newman–Keul's test; $p<0.05$) when the group of animals not submitted to sodium-depletion is compared to all other groups. Each bar in the graph has been obtained from a naive group of animals.

border, the globus pallidus as dorsal limit and the stria terminalis as medial rim. The CeA has four distinct subdivisions: the capsular, the lateral, the intermediate and the medial. The MeA is located near the surface bounded medially by the optic tract and has four subdivisions: rostral, central (dorsal and ventral) and caudal (McDonald, 1998; Sah et al., 2003; Swanson and Petrovich, 1998). The cytoarchitectonic and connectional differences among the discrete subparts of the amygdala correspond to distinct functional roles and selective investigation of the physiology of the central and medial amygdala as separate functional units has been a common practice. The several distinct subnuclei within the amygdala may play specific functional roles in the control of salt intake that have to be continuously explored, in the near future, by the use of techniques and experimental protocols tailored to allow the individualization of those functional differences. Results obtained by pharmacological approaches, as those used here, have to be extended and confirmed by complementary methods such as morphological detection of active neurons during selective ingestive behaviors, as well as anterograde and retrograde axonal labeling designed to identify neural connections involved in those behaviors. Indeed, it was recently demonstrated, by the use of axonal labeling techniques, that CeA is connected to a special group of aldosterone-sensitive neurons in the nucleus of the solitary tract having the lateral parabrachial nucleus as an intermediary relay. This particular pathway may be one of the neuroanatomical circuits explaining the modulation of salt intake by the CeA (Geerling and Loewy, 2006).

The nature of the neurotransmitters related to the mechanisms controlling sodium appetite in the amygdala is unclear. Indeed, we were unable to find studies in which the

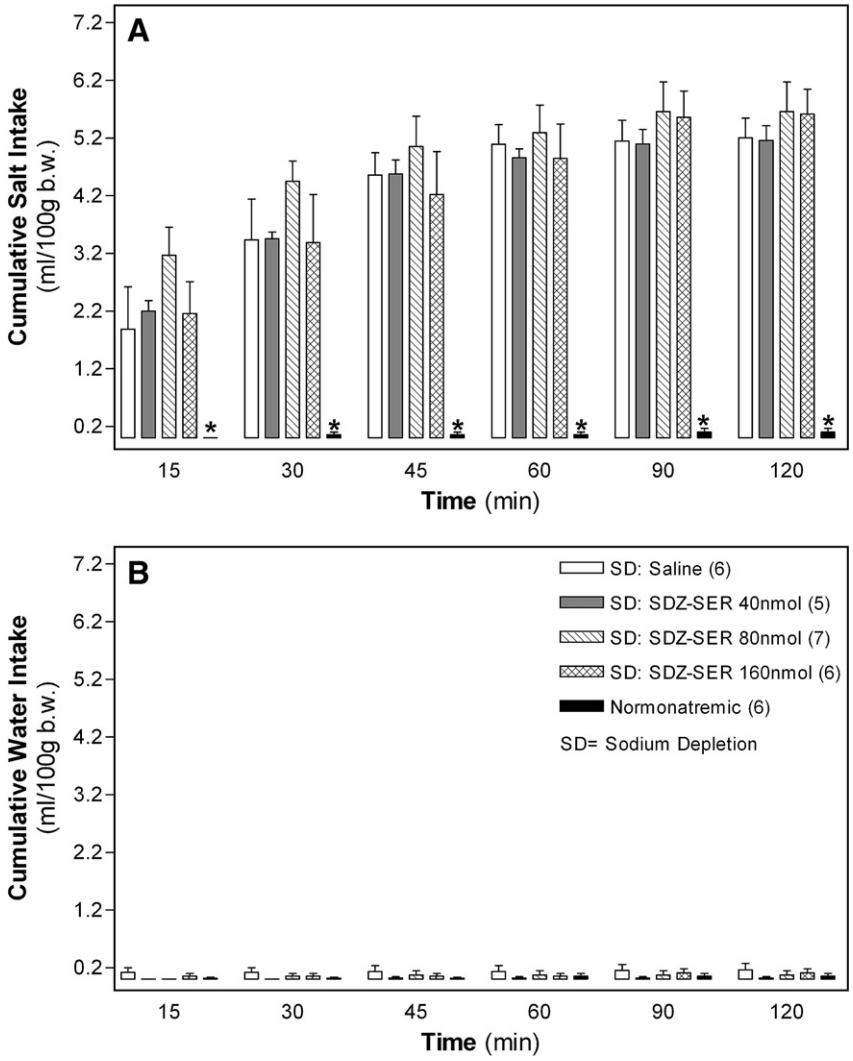

Fig. 9 – Cumulative salt (A) and water (B) intakes (ml/100 g body weight) of sodium-depleted animals treated with bilateral injections of SDZ SER 082, at various doses, into the CeA. The distinct pharmacological treatments and the number of animals used in each group are indicated within panel B. The animals labeled as normonatremic are control animals receiving subcutaneous injections of saline solution, instead of furosemide, and bilateral injections of isotonic saline solution into the CeA. Data are presented as mean ± SEM. Asterisks indicate a statistically significant difference (one-way ANOVA followed by Newman–Keul's test; $p<0.05$) when the group of animals not submitted to sodium-depletion is compared to all other groups. Each bar in the graph has been obtained from a naive group of animals.

aminergic neurochemical mechanisms that modulate water and salt intake in this structure were investigated. The only exception is the finding that cholinergic stimulation of the amygdaloid complex reduces water and salt intake in water-deprived rats (Saad et al., 1994). However, it is important to note that the mineralocorticoid system in the amygdala may play a crucial role in the regulation of fluid balance since genomic and non-genomic effects of mineralocorticoids in this area may influence salt intake. Indeed, the use of antisense oligodeoxynucleotides against mineralocorticoid receptors located within the amygdala reduces DOCA-induced salt intake (Sakai et al., 2000) but have no effect on salt intake elicited by adrenalectomy (Sakai et al., 1996).

In a previous study, we found that the pharmacological activation of 5-HT_3 receptors within the MeA inhibits salt intake and that, in this same brain region, the functional integrity of 5-HT_{2C} receptors is required for the full expression of salt intake in sodium-depleted rats (Luz et al., 2006). Taking in consideration the findings of the present study and data contained in our previous work, it is possible to conclude that the activation of 5-HT_3 receptors located in the MeA and the CeA induces a reduction in salt intake in sodium-depleted rats, revealing a synergistic cooperation between these structures in the control of salt intake. Comparing the results obtained in both studies, it seems that activation of 5-HT_3 receptors within the CeA promotes a more significant antinatriorexic effect compared to the salt intake-inhibiting action that results from the activation of 5-HT_3 receptors located within the MeA. Indeed, the injection of the selective 5-HT_3 receptor agonist m-CPBG at the dose of 160 nmol into the CeA completely abolishes salt intake in sodium-depleted rats, while administration of the same dose

Table 2 – Effect of injections of ondansetron or saline into the CeA in normonatremic animals

Treatment		Time					
	Intakes	15	30	45	60	90	120
Saline (6)	Water	0.03±0.03	0.03±0.03	0.03±0.03	0.06±0.06	0.06±0.06	0.06±0.06
	Salt	0.00±0.00	0.06±0.06	0.06±0.06	0.06±0.06	0.13±0.08	0.13±0.08
Ondansetron (6)	Water	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Salt	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
ANOVA	Water	$F_{(1,10)}=1.0$; $p=0.34$	$F_{(1,10)}=1.0$; $p=0.34$	$F_{(1,10)}=1.0$; $p=0.34$	$F_{(1,10)}=1.0$; $p=0.34$	$F_{(1,10)}=1.0$; $p=0.34$	$F_{(1,10)}=1.0$; $p=0.34$
	Salt	$F_{(1,10)}=1.0$; $p=1.0$	$F_{(1,10)}=1.0$; $p=0.34$	$F_{(1,10)}=1.0$; $p=0.34$	$F_{(1,10)}=1.0$; $p=0.34$	$F_{(1,10)}=2.5$; $p=0.14$	$F_{(1,10)}=2.5$; $p=0.14$

Results are shown as mean±SEM. There were no statistically significant differences among the groups. The number of animals used in each experimental set is indicated in the parenthesis.

of m-CPBG into the MeA significantly inhibits, but does not abolish, salt intake in rats after sodium depletion. Comparison of the data produced in both studies confirms that, in agreement with our findings in the MeA, the administration of the 5-HT_3 receptor antagonist ondansetron into the CeA failed to increase salt intake in normonatremic and sodium-depleted rats, indicating the absence of an endogenous inhibitory tonus exerted by 5-HT_3 receptors in these structures on salt intake. The absence of an endogenous 5-HT_3 receptor-dependent tonus modulating salt intake in the CeA suggests that the inhibitory action of m-CPBG on sodium appetite observed in the present study represents a pharmacological effect of the drug.

The administration of the 5-HT_{2C} agonist m-CPP into the CeA failed to modify water and salt intake in sodium-depleted rats. This result is similar to our previous findings following injection of the same pharmacological agent into the MeA. In that study, the injection of SDZ SER 082, a selective 5-HT_{2C} antagonist, into the MeA elicited a decrease in salt intake in sodium-depleted rats indicating that the endogenous serotonergic activity on 5-HT_{2C} receptors located in this region is necessary for the full expression of sodium appetite in sodium-depleted rats. Conversely, in the present study, the administration of the same 5-HT_{2C} receptor blocker, at the same doses, into the CeA was unable to modify salt intake in sodium-depleted animals, suggesting that 5-HT_{2C} receptors in this region do not participate in the neurochemical mechanisms modulating salt intake in sodium-depleted rats.

The structure of food behavior and the specific appetite for different nutrients is selectively modulated by brain serotonin (Simanski, 1996). Behavioral parameters such as the delay to initiate a meal, the duration of meals and the amount of food intake at each meal are affected by the brain serotonin system. Indeed, fenfluramine, a serotonin releaser, reduces the delay to begin a meal, the speed of eating and the amount of food consumed at each meal (Blundell and Lathan, 1980). In addition, brain serotonin seems to control the selective intake of the distinct nutrients by reducing fat and protein intake without modifying the ingestion of carbohydrates (Smith et al., 1999). The effects of central serotonin systems on sodium appetite are less studied. However, we have previously demonstrated that intracerebroventricular injections of both 5-HT_{2C} and 5-HT_3 receptor agonists inhibit salt intake (Castro et al., 2003). Another group has shown that the blockade of serotonergic action by methysergide at the lateral parabrachial nucleus significantly increases salt intake in rats (Colombari et al., 1996; Menani et al., 1998, 2000). Ingestive behaviors are part of a general reward system that is at least partially controlled by the amygdala, whose functional integrity is essential for the correct choice of food by mammals (Baxter and Murray, 2002).

Several studies reveal that 5-HT_3 receptors are widespread throughout the central nervous system, being present in areas related to the control of hydrosaline balance such as the hypothalamus, the amygdala and the septal area (Tecott et al., 1993). Also, 5-HT_{2C} receptors are universally found in the brain and are present in limbic areas linked to the control of water and salt intake (Barnes and Sharp, 1999; Clement et al., 2000; Giorgetti and Tecott, 2004). Therefore, studies investigating the participation of these receptors located within the amygdala in the control of water and salt intake are based on anatomically proven data.

The serotonergic drugs used in the present study are considered suitable pharmacological agents for studying the role of serotonergic 5-HT_3 and 5-HT_{2C} receptors. Indeed, m-CPBG is a well-known 5-HT_3 receptor agonist (Sepúlveda et al., 1991; Van Hooft and Vijverberg, 1997), the antagonistic effect of ondansetron on 5-HT_3 receptor is a well-documented phenomenon (Gaster and King, 1997), m-CPP is a 5-HT_{2C} agonist (Simansky et al., 2004) and SDZ SER 082 is a specific 5-HT_{2C} receptor antagonist (Hernandez et al., 2003). Despite the fact that m-CPP may exhibit some affinity for other serotonin receptors, it is still considered an ideal tool for the investigation of the physiological roles of 5-HT_{2C} receptors since it binds to this serotonin receptor subtype with much greater affinity than to any other serotonin receptor. Indeed, in the absence of a more selective agonist, m-CPP is considered the prototypical pharmacological tool for the investigation of 5-HT_{2C} function (Hajos et al., 2003; Jakus et al., 2003; Mitchell et al., 2003; Simansky et al., 2004).

The identification of pharmacological effects of serotonergic agents on the central nervous system is of crucial importance since 5-HT_3 receptor antagonists, which easily cross the blood–brain barrier, are the main therapeutical approach to controlling non-coercible nausea and vomiting, generally presented in patients under chemotherapy (Doherty, 1999). Also, central 5-HT_3 receptors may participate in many pharmacological events associated with anxiolytic, antipsychotic and cognitive actions, opening a new field in

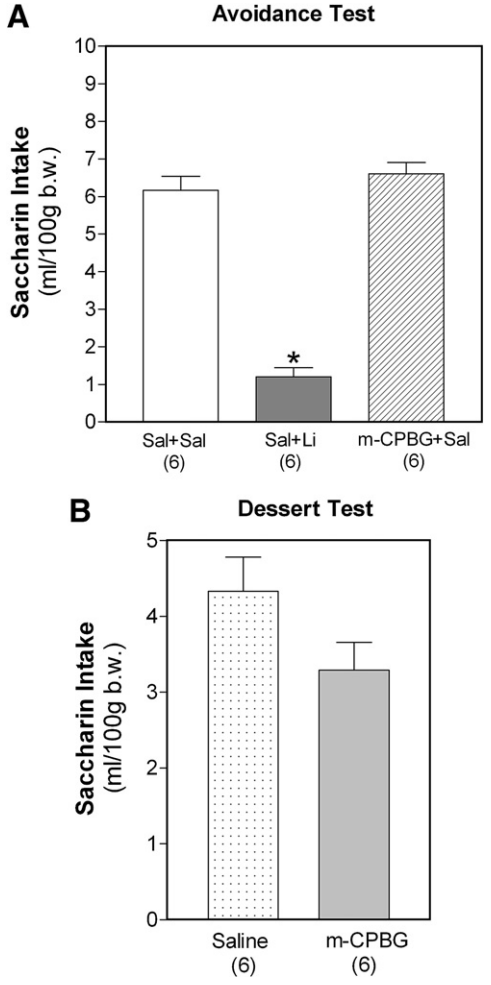

Fig. 10 – Avoidance test: saccharin solution (0.25%) consumption (ml/100 g body weight) over 15 min at a second offering in animals receiving injections of m-CPBG (160 nmol) or saline into the CeA (A). The sequence of injections used during the first offering of saccharin and the number of animals used are indicated in the figure. The first injection was into the CeA and the second via intraperitoneal route. The asterisk indicates a statistically significant difference ($p<0.001$) between that particular group and controls (saline + saline). Dessert test: saccharin intake (ml/100 g body weight) during 2 h in the test cage in rats receiving CeA injections of isotonic saline solution (controls) and m-CPBG at the dose of 160 nmol (B). The treatment received by each group and the number of animals used are indicated in the graph. There was no significant difference in the ingestion of saccharin between groups treated with saline and the serotonergic agent tested. Data are expressed as mean ± SEM.

which 5-HT_3 receptor related compounds may have a future therapeutic use. Therefore, the identification of any pharmacological action of the serotonergic agents used here on important brain functions may be considered relevant.

Inhibition of ingestive behaviors may be the result of aversive effects caused by methodological procedures. In the present study, we have demonstrated that the antinatriorexic effect of m-CPBG is not due to any sickness-like condition since an appropriate test indicated the absence of aversive effects caused by the injection of this drug into the CeA. It is also evident that the injection of m-CPBG does not impair the expression of any hedonic behavior since animals receiving injections of m-CPBG into the CeA normally seek and drink a palatable saccharin solution.

Sodium appetite may be strongly influenced by changes in blood pressure. Sodium intake is more promptly developed during hypovolemia if the animals are made simultaneously hypotensive (Johnson and Thunhorst, 1997). 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 by the use of sympathomimetic drugs such as phenylephrine. This clearly demonstrates the influence of blood pressure in the regulation of sodium appetite (Thunhorst and Johnson, 1994). Furthermore, sodium-depleted sheep display a significant inhibition in salt intake when their blood pressure is maintained elevated (Bott et al., 1967) and baroreceptor denervation significantly decreases sodium appetite in rats after sodium depletion (Thunhorst et al., 1994). These findings indicate that acute increases in blood pressure inhibit sodium appetite. In summary, hypotension seems to stimulate whereas hypertension inhibits salt intake. In the present study, the pharmacological stimulation of 5-HT_3 receptors located within the CeA was unable to modify blood pressure in rats submitted to the same experimental sodium depletion protocol used to study salt intake. This allows the conclusion that the antidipsogenic effect evoked by the injections of m-CPBG into the CeA is not consequent to an increase in blood pressure.

In this study, injections of m-CPBG into areas located outside the CeA did not produce any significant effect on salt intake in sodium-depleted rats, indicating that the effects observed here are consequent to the pharmacological stimulation of 5-HT_3 receptors located within that brain region.

Considering the close proximity between CeA and MeA, we cannot exclude that the drugs injected into each one of these regions may partially reach the other, leading to an overall effect that may represent much more a preferential activation of one of these sites than an exclusive activation of that particular brain region. Therefore, we cannot exclude that, in our previous paper, the inhibitory effect of m-CPBG injections into the MeA on salt intake may result from the action of the drug partially reaching the CeA.

In summary, in this study, we show that pharmacological activation of 5-HT_3 receptors located in the CeA induces a significant decrease in salt intake in sodium-depleted rats. Our findings also suggest that 5-HT_{2C} receptors located in this same region do not participate in the mechanisms controlling salt intake at least in sodium-depleted rats.

4. Experimental procedures

4.1. *Animals*

In the present study, we used male Wistar rats weighing 280 ± 20 g. They were housed in individual cages and kept

Table 3 – Values of F, df and p for the effects obtained after the injections of the serotonergic agents administered alone or in combination, into the CeA, in the experimental sets designed to study the role of brain 5-HT$_3$ and 5-HT$_{2C}$ receptors on water and salt intake in sodium-depleted rats

Treatment compared		Time (min)					
		15	30	45	60	90	120
Saline; m-CPBG (10, 40, 80, 160 nmol) normonatremic	Water	$F_{(5,39)}=1.10$; $p=0.38$	$F_{(5,39)}=1.10$; $p=0.38$	$F_{(5,39)}=1.10$; $p=0.38$	$F_{(5,39)}=1.10$; $p=0.38$	$F_{(5,39)}=2.18$; $p=0.08$	$F_{(5,39)}=0.77$; $p=0.57$
	Salt	$F_{(5,39)}=15.3$; $p<0.0001$	$F_{(5,39)}=14.1$; $p<0.0001$	$F_{(5,39)}=27.3$; $p<0.0001$	$F_{(5,39)}=28.8$; $p<0.0001$	$F_{(5,39)}=30.9$; $p<0.0001$	$F_{(5,39)}=40.2$; $p<0.0001$
Saline + saline; saline + m-CPBG 160 nmol; ondansetron 160 nmol + m-CPBG 160 nmol normonatremic	Water	$F_{(3,28)}=0.67$; $p=0.58$	$F_{(3,28)}=0.72$; $p=0.55$	$F_{(3,28)}=1.76$; $p=0.18$	$F_{(3,28)}=1.76$; $p=0.18$	$F_{(3,28)}=1.76$; $p=0.18$	$F_{(3,28)}=1.40$; $p=0.26$
	Salt	$F_{(3,28)}=15.7$; $p<0.0001$	$F_{(3,28)}=23.6$; $p<0.0001$	$F_{(3,28)}=55.3$; $p<0.0001$	$F_{(3,28)}=67.0$; $p<0.0001$	$F_{(3,28)}=82.2$; $p<0.0001$	$F_{(3,28)}=92.5$; $p<0.0001$
Saline; ondansetron (40, 80, 160 nmol) normonatremic	Water	$F_{(4,29)}=0.85$; $p=0.50$	$F_{(4,29)}=0.85$; $p=0.50$	$F_{(4,29)}=0.85$; $p=0.50$	$F_{(4,29)}=0.80$; $p=0.53$	$F_{(4,29)}=0.80$; $p=0.53$	$F_{(4,29)}=0.80$; $p=0.53$
	Salt	$F_{(4,29)}=3.54$; $p=0.02$	$F_{(4,29)}=9.32$; $p<0.0001$	$F_{(4,29)}=11.2$; $p<0.0001$	$F_{(4,29)}=31.4$; $p<0.0001$	$F_{(4,29)}=49.8$; $p<0.0001$	$F_{(4,29)}=49.4$; $p<0.0001$
Saline m-CPP (40, 80, 160 nmol) normonatremic	Water	$F_{(4,31)}=1.0$; $p=1.0$	$F_{(4,31)}=2.77$; $p=0.05$	$F_{(4,31)}=2.77$; $p=0.05$	$F_{(4,31)}=1.42$; $p=0.25$	$F_{(4,31)}=0.81$; $p=0.53$	$F_{(4,31)}=0.81$; $p=0.53$
	Salt	$F_{(4,31)}=4.79$; $p=0.004$	$F_{(4,31)}=11.0$; $p<0.0001$	$F_{(4,31)}=30.5$; $p<0.0001$	$F_{(4,31)}=42.0$; $p<0.0001$	$F_{(4,31)}=42.9$; $p<0.0001$	$F_{(4,31)}=44.5$; $p<0.0001$
Saline SDZ-SER082 (40, 80, 160 nmol) normonatremic	Water	$F_{(4,26)}=1.23$; $p=0.32$	$F_{(4,26)}=1.15$; $p=0.36$	$F_{(4,26)}=1.19$; $p=0.34$	$F_{(4,26)}=1.14$; $p=0.36$	$F_{(4,26)}=1.16$; $p=0.35$	$F_{(4,26)}=1.13$; $p=0.36$
	Salt	$F_{(4,26)}=5.42$; $p=0.003$	$F_{(4,26)}=10.5$; $p<0.0001$	$F_{(4,26)}=19.6$; $p<0.0001$	$F_{(4,26)}=32.2$; $p<0.0001$	$F_{(4,26)}=44.0$; $p<0.0001$	$F_{(4,26)}=45.5$; $p<0.0001$
m-CPBG (40, 80, 160 nmol) into CeA; m-CPBG (40, 80, 160 nmol) into MeA	Salt	$F_{(5,38)}=11.40$; $p<0.0001$	$F_{(5,38)}=17.70$; $p<0.0001$	$F_{(5,38)}=14.3$; $p<0.0001$	$F_{(5,38)}=17.80$; $p<0.0001$	$F_{(5,38)}=36.2$; $p<0.0001$	$F_{(5,38)}=35.6$; $p<0.0001$

The data were analyzed using one-way ANOVA for each time point. For follow-up statistical tests to compare specific groups, additional post hoc Student–Newman–Keuls tests were conducted. The group means for the various parameters analyzed were considered to be significantly different when $p<0.05$. These results are shown in Figs. 2–6.

under controlled light (lights on from 7 AM to 7 PM) and temperature (22–24 °C) conditions. Central injections of saline (controls) and each individual dose of the serotonergic agents were tested in a naive group of animals. All experiments were conducted between 7 AM and 12 PM. The experimental protocols were conducted according to the regulations established by the National Institutes of Health (USA) and were approved by a local committee regulating the use of animals in research laboratories.

4.2. *Surgical procedures*

Five days before the experimental sessions, a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) was used to implant a 15 mm, 22-gauge, stainless steel cannula under pentobarbital anesthesia (50 mg/kg i.p.). The following coordinates were used: anteroposterior = 2.0 mm behind bregma; lateral = 3.6 mm; vertical = 7.6 mm below the skull. The animals were placed in the stereotaxic apparatus with their heads in the horizontal position. The cannulas were cemented to the skull bone with dental acrylic. To avoid obstruction, an obturator (28-gauge) was provided. After surgery, the animals in all the study groups had free access to two different bottles, one containing distilled water and the other containing 1.5% saline solution. The animals were handled every day in order to minimize the stress of the experimental procedure. At the end of the experiments, the animals were anesthetized with ether and submitted to transcardiac perfusion with isotonic saline solution, followed by 10% formalin. The brains were then removed and fixed in 10% formalin. They were frozen and cut into 40 μm sections. To confirm the injection sites in relation to the CeA, the slices were stained with cresyl violet and analyzed by light microscopy. The data from the animals in which the cannulas were strictly inside the CeA were analyzed and taken into consideration for the interpretation of the effects of the pharmacological agents on water and salt intake. A special table condenses the data from animals in which the cannulas were off target.

4.3. *Drugs and microinjections*

The following drugs were used: *m*-chlorophenylbiguanide hydrochloride (1-(3-cholrophenyl)biguanide; m-CPBG), a selective 5-HT$_3$ agonist (Sepúlveda et al., 1991; Van Hooft and Vijverberg, 1997); m-CPP (1-(3-Chlorophenyl)piperazine), a 5-HT$_2$ agonist (Simansky et al., 2004); and SDZ SER 08 [(+)-cis-4, 5, 7a,8, 9, 10, 11, 11a-octahydro-7*H*-10-methylindolo [1,7-bc][2,6] naphthyridine], a selective 5-HT$_{2C}$ receptor antagonist (Hernandez et al., 2003), all purchased from Tocris Cookson, Inc. Ballwin, MO. Ondansetron, a specific 5-HT$_3$ antagonist, was kindly donated by GlaxoWellcome Research and Development Limited, UK (Gaster and King, 1997). Lithium chloride was acquired from Sigma Chemical, Co., St. Louis, MO. Furosemide, a loop diuretic, was purchased from Aventis Pharma Ltd., São Paulo, Brazil. Central injections were performed using Hamilton microsyringes connected to Myzzy–Slide–Pak needles through polyethylene tubing. The injectors we have used extended 1 mm beyond the end of the guide cannulas. All drugs were dissolved in isotonic saline solution. The final volume injected was 0.5 μl over a period of 60 seconds.

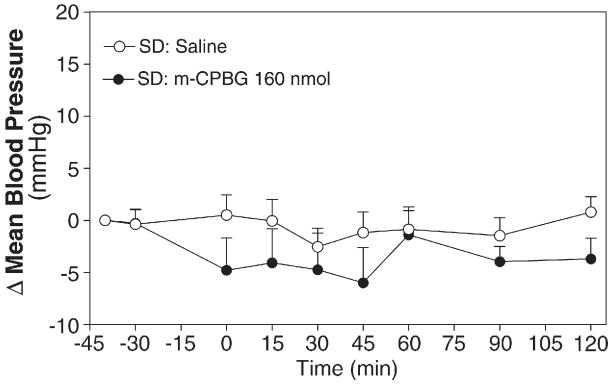

Fig. 11 – Changes in mean blood pressure in sodium-depleted rats after injections of m-CPBG (● 160 nmol; *n*=6) or saline solution (○; *n*=6), into the CeA. Blood pressure recording between times −45 and −30 min corresponds to a pre-drug basal evaluation. Intra-amygdaloid injections were made at time −30 min. The bottles containing water and saline solution (1.5%) were accessible at 0 min. Data are presented as mean±SEM.

4.4. *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 CeA cannulation until the moment of furosemide administration. To provoke the renal sodium loss that induces sodium depletion, the rats received a subcutaneous injection of furosemide (20 mg/kg) 24 h prior to the experimental sessions. Access to 1.5% saline ceased immediately after the furosemide injection. From that moment on, 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^+). Control animals not submitted to sodium depletion received subcutaneous injections of isotonic saline solution instead of furosemide. We have previously shown that furosemide administration, at the dose used here, effectively increases urine output and renal sodium excretion and produces hyponatremia (Castro et al., 2003). To test the participation of central 5-HT_{2C} and 5-HT_3 receptors in water and salt intake in sodium-depleted rats, different groups of sodium-depleted animals received bilateral injections of the serotonergic agents at different doses into the CeA. Sodium-depleted control animals received injections of isotonic saline solution into this same area. The bottles containing 1.5% saline solution were reintroduced into the cages 30 min after the injections into the CeA. The first measurement of fluid intake was recorded 15 min after this and measurements continued for the next 120 min. All groups were also compared to a control group of normonatremic animals.

4.5. *Avoidance test*

An avoidance test was carried out to verify whether the central administration of the serotonergic agent, m-CPBG, was devoid of non-specific, inhibitory, "illness-like" effects on salt intake. An experimental protocol based on the original design proposed by Nachman (1970) was adopted. This protocol uses a temporal association between the novel taste of a 0.25% saccharin solution and the distress induced by lithium chloride administration. Five days after cannulation of the CeA, the animals had their access to water restricted to 15 min/day (between 12 and 12:15 PM) for 4 consecutive days. Under these conditions, rats drank water rapidly and reliably. On the fifth day, they were divided into 3 different groups that, after being submitted to the different pharmacological protocols, had access to bottles containing saccharin (no water was offered on this day). The first group (controls) received two consecutive injections of isotonic saline solution, one immediately following the other, the first being intraperitoneal and the second into the CeA. In the second group of animals, 0.15 M lithium chloride intraperitoneal injections (0.6% b.w.) were followed by injections of isotonic saline solution into the CeA. In this group, the lithium-induced, illness-like effects, a condition that generally disrupts ingestive behaviors in rats, were associated with the novel taste of saccharin. The third group of animals received intraperitoneal injections of saline solution in the same volume used in the previous group, followed by injections of m-CPBG (160 nmol). In this group of animals, we investigated whether the administration of the serotonergic agent m-CPBG into the CeA provoked any degree of discomfort leading to a general reduction in ingestive behavior that the animals could associate with the novel taste of saccharin. On the sixth day, at the same time that the bottles had been available on the previous days (12 to 12:15 PM), saccharin-containing bottles were placed in all cages and the amount ingested was recorded. No drugs were injected on this day.

4.6. *Dessert test*

To investigate whether the serotonergic agents used in the present study were able to modify water and salt intake through non-specific, general inhibition of the central nervous system or through a locomotor deficit, we investigated the effect of their injection into the CeA on the intake of 0.1% saccharin solution, a well-established example of hedonic behavior in rats (Johnson and Schwob, 1975). In this experiment, after CeA cannulation, two different groups of animals, kept in the usual individual cages where the only fluid available was water, were transferred (for 2 h each day for seven consecutive days) to a different cage (the test cage) in which two bottles, one containing water and the other containing a 0.1% saccharin solution, were accessible. After this period of training, two different groups of fluid-deprived animals received injections of m-CPBG (160 nmol) or saline (controls) into the CeA, 30 min before being transferred to the test cage. The intake of saccharin was then recorded during the following 120 min.

4.7. *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.

To test the effects of the intra-amygdaloid administration of m-CPBG on blood pressure, distinct groups of sodium-depleted animals, whose blood pressure had already been monitored for 15 min, received injections of m-CPBG (160 nmol) or saline solution (controls) into the CeA. In each of those groups, blood pressure continued to be recorded for the next 150 min after m-CPBG or saline solution was injected. The experimental protocol used in this case was identical to that used to study salt intake in the previous groups.

4.8. *Statistical analysis*

A computer software package (SigmaStat for Windows, Jandel Scientific, San Rafael-CA) was used to carry out a one-way analysis of variance for each time point. The post hoc Student–Newman–Keuls test was used for comparison of each treatment with its corresponding time in the control groups. One-way ANOVA was also used to analyze the data resulting from the avoidance test. Two-way ANOVA was used to analyze the data in the experimental set designed to investigate the effect of intra-amygdaloid injections of m-CPBG on blood pressure. Data resulting from the dessert test were analyzed using Student's *t*-test. The data are presented as mean ± SEM. The effects were considered significantly different when $p < 0.05$.

Acknowledgments

We are grateful to Mr. Vanilson Souza and Mr. José de Souza for their skillful technical assistance. The present work was supported by grants provided by The Brazilian Council of Research (CNPq), grant numbers 300.915/2003-9 and 300.943/2003-2, and by The Financial Agency for the Support of Research of the State of Bahia (FAPESB). Carla Luz received financial support from the State University of Southwest Bahia.

REFERENCES

Barnes, N.M., Sharp, T., 1999. A review of central 5-HT receptors and their function. Neuropharmacology 38, 1083–1152.

Baxter, M.G., Murray, E.A., 2002. The amygdala and reward. Nat. Rev., Neurosci. 3, 563–573.

Blundell, J.E., Lathan, C.J., 1980. Characterization of adjustments to the structure of feeding behavior following pharmacological treatment: effects of amphetamine and fenfluramine and antagonism produced by pimozide and methergoline. Pharmacol. Biochem. Behav. 12, 717–722.

Bott, E., Denton, D.A., Weller, S., 1967. The effect of angiotensin II infusion, renal hypertension and nephrectomy on salt appetite of sodium-deficient sheep. Aust. J. Exp. Biol. Med. Sci. 45, 595–612.

Castro, L., De Castro e Silva, E., Lima, A.K.S., Souza, F.S., Maldonado, I., Macedo, D.F., Ferreira, M.G., Santamaría, G.F., Bandeira, I.P.V., Amor, A.L.M., Carvalho, F.L.Q., Rocha Jr., M.A., Oliveira, I.R., Fregoneze, J.B., 2000. Central 5-HT_4 receptors and drinking behavior. Pharmacol. Biochem. Behav. 66, 443–448.

Castro, L., Maldonado, I., Campos, I., Varjão, B., Ângelo, A.L., Athanazio, R.A., Barbetta, M.C., Ramos, A.C., Fregoneze, J.B., De Castro e Silva, E., 2002a. Central administration of m-CPP, a serotonin 5-$HT2_B/2_C$ agonist, decreases water intake in rats. Pharmacol. Biochem. Behav. 72, 891–898.

Castro, L., Varjão, B., Maldonado, I., Campos, I., Duque, B., Fregoneze, J.B., Oliveira, I.R., De Castro e Silva, E., 2002b. Central 5-HT_3 receptors and water intake in rats. Physiol. Behav. 77, 349–359.

Castro, L., Athanazio, R., Barbetta, M., Ramos, A.C., Ângelo, A.L., Campos, I., Varjão, B., Ferreira, H.S., Fregoneze, J.B., De Castro e Silva, E., 2003. Central 5-HT2B/2C and 5-HT_3 receptor stimulation decreases salt intake in sodium-depleted rats. Brain Res. 981, 151–159.

Clement, D.A., Punhani, T., Duxon, M.S., Blackburn, T.P., Fone, K.C., 2000. Immunohistochemical localization of the 5-HT_{2C} receptor protein in the rat CNS. Neuropharmacology 39, 123–132.

Colombari, D.S., Menani, J.V., Johnson, A.K., 1996. Forebrain angiotensin type I receptors and parabrachial serotonin in the control of NaCl and water intake. Am. J. Physiol. 271, R1470–R1476.

De Castro e Silva, E., Sarmento, C., Nascimento, T.A., Luz, C.P., Soares, T., Marinho, A., Cunha, M., Bulcão, C., De Oliveira, I.R., Fregoneze, J.B., 1997. Effect of third ventricle administration of L-694-247, a selective 5-$HT1_D$ receptor agonist, on water intake in rats. Pharmacol. Biochem. Behav. 57, 749–754.

Doherty, K.M., 1999. Closing the gap in prophylactic antiemetic therapy: patient factors in calculating the emetogenic potential of chemotherapy. Clin. J. Oncol. Nurs. 3, 113–119.

Galaverna, O., De Luca Jr., L.A., Schulkin, J., Yao, S.Z., Epstein, A.N., 1992. Deficits in NaCl ingestion after damage to the central nucleus of the amygdala in the rat. Brain Res. Bull. 28, 89–98.

Gaster, L.M., King, F.D., 1997. Serotonin 5-HT_3 and 5-HT_4 receptor antagonists. Med. Res. Rev. 17, 163–214.

Geerling, J.C., Loewy, A.D., 2006. Aldosterone-sensitive neurons in the nucleus of the solitary tract: bidirectional connections with the central nucleus of the amygdala. J. Comp. Neurol. 497, 646–657.

Giorgetti, M., Tecott, L.H., 2004. Contributions of 5-HT(2C) receptors to multiple actions of central serotonin systems. Eur. J. Pharmacol. 488, 1–9.

Hajos, M., Hoffmann, W.E., Weaver, R.J., 2003. Regulation of septo-hippocampal activity by 5-hydroxytryptamine(2C) receptors. J. Pharmacol. Exp. Ther. 306, 605–615.

Hernandez, M., Barahona, M.V., Simonsen, U., Recio, P., Rivera, L., Martinez, A.C., Garcia-Sacristan, A., Orensanz, L.M., Prieto, D., 2003. Characterization of the 5-hydroxytryptamine receptors mediating contraction in the pig isolated intravesical ureter. Br. J. Pharmacol. 138, 137–144.

Jakus, R., Graf, M., Juhasz, G., Gerber, K., Levay, G., Halasz, P., Bagdy, G., 2003. 5-HT_{2C} receptors inhibit and 5-HT_{1A} receptors activate the generation of spike-wave discharges in a genetic rat model of absence epilepsy. Exp. Neurol. 184, 964–972.

Johnson, A.K., Schwob, J.E., 1975. Cephalic angiotensin receptors mediating drinking to systemic angiotensin II. Pharmacol. Biochem. Behav. 3, 1077–1084.

Johnson, A.K., Thunhorst, R.L., 1997. The neuroendocrinology of thirst and salt appetite: visceral sensory signals and mechanisms of central integration. Front. Neuroendocrinol. 18, 292–353.

Johnson, A.K., De Olmos, J., Pastuskovas, C.V., Zardetto-Smith, A.M., Vivas, L., 1999. The extended amygdala and salt appetite. Ann. N. Y. Acad. Sci. 877, 258–280.

Luz, C., Souza, A., Reis, R., Fregoneze, J.B., De Castro e Silva, E., 2006. Role of 5-HT_3 and 5-HT_{2C} receptors located within the medial amygdala in the control of salt intake in sodium-depleted rats. Brain Res. 1099, 121–132.

McDonald, A.J., 1998. Cortical pathways to the mammalian amygdala. Prog. Neurobiol. 55, 257–332.
Menani, J.V., De Lucca Jr., L.A., Johnson, A.K., 1998. Lateral parabrachial nucleus serotonergic mechanisms and salt appetite induced by serotonergic mechanisms and salt appetite induced by sodium depletion. Am. J. Physiol. 274, R555–R560.
Menani, J.V., De Lucca Jr., L.A., Thunhorst, R.L., Johnson, A.K., 2000. Hindbrain serotonin and the rapid induction of sodium appetite. Am. J. Physiol. 279, R126–R131.
Mitchell, P.J., Fairhall, S.J., Fletcher, A., Redfern, P.H., 2003. Effects of single and repeated electroconvulsive shock on the social and agonistic behavior of resident rats. Neuropharmacology 44, 911–925.
Nachman, M., 1970. Learned taste and temperature aversions due to lithium chloride sickness after temporal delays. J. Comp. Physiol. Psychol. 73, 22–30.
Nachman, M., Ashe, J.H., 1974. Effects of basolateral amygdala lesions on neophobia, learned taste aversions, and sodium appetite in rats. J. Comp. Physiol. Psychol. 87, 622–643.
Nitabach, M.N., Schulkin, J., Epstein, A.N., 1989. The medial amygdala is a part of a mineralocorticoid-sensitive circuit controlling NaCl intake in the rat. Behav. Brain Res. 35, 127–134.
Price, J.L., Russche, F.T., Amaral, D.G., 1987. In: Björklund, A., Hökfelt, T., Swanson, L.W. (Eds.), Handbook of Chemical Neuroanatomy. . Integrated Systems of the CNS, Part 1, vol. 5. Elsevier, Amsterdam, pp. 279–388.
Saad, W.A., Bengtson, R.M., Menani, J.V., Camargo, L.A., Renzi, A., Silveira, J.E., Saad, W.A., 1994. Effect of cholinergic stimulation of the amygdaloid complex on water and salt intake. Braz. J. Med. Biol. Res. 27, 915–920.
Sah, P., Faber, E.S.L., Lopez de Armentia, M., Power, J., 2003. The amygdaloid complex: anatomy and physiology. Physiol. Rev. 83, 803–834.
Sakai, R.R., Ma, L.Y., Zhang, D.M., McEwen, B.S., Fluharty, S.J., 1996. Intracerebral administration of mineralocorticoid receptor antisense oligonucleotides attenuate adrenal steroid-induced salt appetite in rats. Neuroendocrinology 64, 425–429.
Sakai, R.R., McEwen, B.S., Fluharty, S.J., Ma, L.Y., 2000. The amygdala: site of genomic and non-genomic arousal of aldosterone-induced sodium intake. Kidney Int. 57, 1337–1345.
Schulkin, J., Marini, J., Epstein, A.N., 1989. A role for the medial region of the amygdala in mineralocorticoid-induced salt hunger. Behav. Neurosci. 103, 178–185.
Sepúlveda, M.I., Lummis, S.C., Martin, I.L., 1991. 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, 536–540.
Simanski, K.J., 1996. Serotonergic control of the organization of feeding and satiety. Behav. Brain Res. 73, 37–42.
Simansky, K.J., Dave, K.D., Inemer, B.D., Nicklous, D.M., Padron, J.M., Aloyo, V.J., Romano, A.G., 2004. A 5-HT_{2C} agonist elicits hyperactivity and oral dyskinesia with hypophagia in rabbits. Physiol. Behav. 82, 97–107.
Smith, B.K., York, D.A., Bray, G.A., 1999. Activation of hypothalamic serotonin receptors reduced intake of dietary fat and protein but not carbohydrate. Am. J. Physiol. 277, R802–R811.
Swanson, L.W., Petrovich, G.D., 1998. What is the amygdala? Trends Neurosci. 21, 323–331.
Tecott, L.H., Maricq, A., Julius, V.D., 1993. Nervous system distribution of the serotonin 5-HT_3 receptor mRNA. Proc. Natl. Acad. Sci. 90, 1430–1434.
Thunhorst, R.L., Johnson, A.K., 1994. Renin–angiotensin, arterial blood pressure and salt appetite in rats. Am. J. Physiol. 266, R458–R465.
Thunhorst, R.L., Lewis, S.J., Johnson, A.K., 1994. Effects of sinoaortic baroreceptor denervation on depletion-induced salt appetite. Am. J. Physiol. 267, R1043–R1049.
Uphouse, L., 1997. Multiple serotonin receptors: too many, not enough, or just the right number? Neurosci. Biobehav. Rev. 21, 679–698.
Van Hooft, J.A., Vijverberg, H.P., 1997. Full and partial agonists induce distinct desensitized states of the 5-HT_3 receptor. J. Recept. Signal Transduction Res. 17, 267–277.
Woodwar, D.J., Moises, H.C., Waterhouse, B.D., Hoffer, B.J., Freedman, R., 1979. Modulatory actions of norepinephrine in the central nervous system. Fed. Proc. 38, 2109–2116.
Zardetto-Smith, A.M., Beltz, T.G., Johnson, A.K., 1994. Role of central nucleus of the amygdala and bed nucleus of stria terminalis in experimentally-induced salt appetite. Brain Res. 645, 123–134.
Zhang, D.M., Epstein, A.N., Schulkin, J., 1993. Medial region of the amygdala: involvement in adrenal-steroid-induced salt appetite. Brain Res. 600, 20–26.