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Central administration of *m*CPP, a serotonin 5-$HT_{2B/2C}$ agonist, decreases water intake in rats

L. Castro[a], I. Maldonado[a], I. Campos[a], B. Varjão[a], A.L. Angelo[a], R.A. Athanazio[a], M.C. Barbetta[a], A.C. Ramos[a], J.B. Fregoneze[b], E. De Castro e Silva[a,*]

[a]*Department of Physiology, Health Sciences Institute, Federal University of Bahia, 40110-100, Salvador, Bahia, Brazil*
[b]*Department of Zoology, Biology Institute, Federal University of Bahia, 401170-110, Salvador, Bahia, Brazil*

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Abstract

In the present study, we investigated in rats the effect of third ventricle injections of 1-(3-chlorophenyl)piperazine (*m*CPP), a 5-HT_2 receptor agonist, on water intake induced by three different physiological stimuli: fluid deprivation, acute salt load and hypovolemia. Injections of *m*CPP in the doses of 80 and 160 nmol/rat were able to decrease water intake in all three conditions studied. Third ventricle injections of *m*CPP (160 nmol/rat) were no longer able to diminish water intake in the groups of rats pretreated with central injections of an equimolar amount of (+)-cis-4,5,7a,8,9,10,11,11a-octahydro-7*H*-10-methylindolo[1,7-bc][2,6]-naphthyridine (SDZ SER 082), a selective 5-$HT_{2B/2C}$ antagonist. The central administration of *m*CPP (160 nmol/rat) was not able to modify the intake of a 0.1% saccharin solution. It is suggested that the central activation of a 5-$HT_{2B/2C}$ component is able to impair the drive for water intake induced by the physiological stimuli represented by fluid deprivation, acute salt load and hypovolemia. This effect seems not to be consequent on a general nonspecific central nervous system depression or on a locomotor deficit, because saccharin intake is not affected by third ventricle injections of *m*CPP.

Keywords: 5-$HT_{2B/2C}$ receptors; Drinking behavior; Hypovolemia; Hyperosmolarity; Fluid deprivation; Serotonin; *m*CPP

1. Introduction

The central serotonergic component, a chemically and anatomically well-defined brain neurotransmitter system operating several families of serotonin receptors, exerts multiple physiological actions and is pharmacologically modified by a multitude of exogenous agents (Barnes and Sharp, 1999). It is well known that the central serotonin system plays a prominent role in the control of feeding behavior (Leibowitz and Alexander, 1998), but brain serotonin effects on water and salt intake have been much less studied. Nevertheless, it seems that central serotonin pathways may induce an inhibitory effect on water intake (Neil and Cooper, 1989a; Shisheva et al., 1987). We have previously shown that water intake induced by dehydration or by pharmacological activation of central cholinergic, β-adrenergic and angiotensinergic components in rats is inhibited by pharmacological activation of central postsynaptic 5-HT_{1D} receptors (De Castro-e-Silva et al., 1997). More recently, we demonstrated that central 5-HT_4 receptors seem to exert a dual effect on water intake, inhibiting thirst after central cholinergic activation and potentiating drinking due to central angiotensinergic stimulation (Castro et al., 2000).

Among the distinct serotonin receptors, the 5-HT_2 receptor family comprises three different subtypes (5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}) whose molecular structure, pharmacology and signal transduction pathways share common aspects (Barnes and Sharp, 1999). Particular attention has been recently devoted to the central 5-HT_2 receptor component, because drugs acting on central 5-HT_2 receptors are currently being assessed as potential agents in the treatment of several disorders including schizophrenia, anxiety, depression and migraine (Baxter et al., 1995).

The different physiological conditions related to the body fluid and electrolyte changes yield distinct inputs that reach the central nervous system. These inputs influence a com-

* Corresponding author. Departamento de Biorregulação, Instituto de Ciências da Saúde, Universidade Federal da Bahia, 40110-100 Salvador - BA, Brazil. Tel.: +55-71-235-7518; fax: +55-71-337-0591.
E-mail address: emilio@svn.com.br (E. De Castro e Silva).

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plex network interconnecting different brain areas and neurotransmitter pathways whose final output to the higher integrative circuitry regulates drinking behavior (Johnson and Thunhorst, 1997).

In the present paper, we use a pharmacological approach to investigate the role of central 5-HT_2 receptors on water intake during different thirst-inducing physiological conditions such as fluid deprivation, hyperosmolarity and hypovolemia.

2. Method

2.1. Animals

Adult Wistar male rats weighing 240 ± 20 g kept under controlled light (lights on from 07:00 to 19:00 h) and temperature (22–24 °C) conditions were used in the experiments. The animals had free access to water and laboratory chow (Nuvital Nutrientes, Curitiba, Brazil) in the days before the experiments. They were handled daily, and the groups receiving intragastric salt load during the experimental session were previously habituated to the introduction of an oral intragastric tube, every 2 days. The experimental protocols were conducted according to the rules suggested by the National Institute of Health (USA) as required by the Federal University of Bahia Research Board.

2.2. Surgical procedure

Seven days before the experimental sessions, the third ventricle was cannulated under pentobarbital anesthesia (40 mg/kg ip). A 22-G stainless steel cannula (15 mm in length) was stereotaxically implanted according to the following coordinates: anteroposterior = 0.5 mm behind bregma, lateral = precisely on the middle line and vertical = 8.5 mm below the skull. The cannulas were cemented to the skull with dental acrylic and provided with an obturator (30 G). The animals were positioned in the stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with the head inclined +0.2 mm upward, avoiding lesions to structures related to body fluid and electrolyte control. After the surgery, the animals were housed for 7 days in the same type of individual cages in which they were monitored during the experiments.

After sacrifice by CO_2 inhalation, a third ventricle injection of Blue Evans dye was performed in order to confirm whether the tip of the cannula was correctly positioned. Only the data from animals in which the cannula tip was clearly located within the third ventricle were included in subsequent analysis.

2.3. Drugs and injections

1-(3-Chlorophenyl)piperazine (*m*CPP), a 5-HT_2 agonist (Kahn and Wetzler, 1991), and (+)-cis-4,5,7a,8,9,10,11,11a-octahydro-7*H*-10-methylindolo[1,7-bc][2,6]-naphthyridine (SDZ SER 082), a selective 5-$HT_{2B/2C}$ receptor antagonist (Willins and Meltzer, 1997), were purchased from Tocris Cookson, Ballwin, MO. Polyethylene glycol (PEG) (mw 15.000–20.000) was acquired from Sigma, St. Louis, MO.

A 30% PEG solution used to induce hypovolemia was prepared in 0.15-M sodium chloride by heating the mixture to approximately 50 °C while it was being constantly stirred. This solution was subcutaneously administered (2 ml/100 g body weight). SDZ SER 082 and *m*CPP were dissolved in isotonic saline solution and injected into the third ventricle using a Hamilton microsyringe connected to a 30-G injector through a polyethylene tubing (no. 10). A total volume of 2 μl was injected over 60 s.

2.4. Experimental design

We investigated the role of central 5-HT_2 receptors in water intake in three different thirst-inducing physiological conditions: fluid deprivation, hyperosmolarity and hypovolemia. To achieve fluid deprivation, the access of the animals to water was restricted for 14 h in the overnight period immediately prior to the experiments (from 06:00. to 08:00 h). To test the effect of *m*CPP on water intake after fluid deprivation, fluid-deprived animals received one of the three doses of *m*CPP (40, 80 and 160 nmol/rat) or isotonic saline (controls) 30 min before the reintroduction of the graduated bottles into the cages. To study the participation of central 5-$HT_{2B/2C}$ receptors in the effects of *m*CPP, a group of fluid-deprived animals treated with third ventricle injections of *m*CPP in the dose of 160 nmol/rat was pretreated with third ventricle injections of SDZ SER 082, a selective 5-$HT_{2B/2C}$ receptor antagonist, in equimolar amount or isotonic saline (controls) 10 min before receiving *m*CPP. In both cases, water intake was recorded for the next 120 min following the last injection. These groups were also compared to an additional group of animals not submitted to fluid deprivation and receiving third ventricle injections of isotonic saline.

The animals used to study the participation of central 5-HT_2 receptors in water intake induced by hyperosmolarity were fasted for 14 h, from 06:00 to 08:00 h, the night before the experiments. Ten minutes after the third ventricle injections of *m*CPP (40, 80 and 160 nmol/rat) or isotonic saline solution (controls), the animals received an intragastric salt load. This was achieved by administrating 1 ml/100 g body weight of a hypertonic saline solution (1.5 M) via orogastric tubing. Twenty minutes after the salt load, water-containing graduated bottles were reintroduced into the cages and the cumulative water intake was recorded over the next 120 min. In this experimental set, the graduated bottles were removed from the cages immediately before the intracerebroventricular injections, being reintroduced 30 min after. To test the participation of central 5-$HT_{2B/2C}$ receptors in the effects of *m*CPP in water intake induced by hyperosmolarity, a group of salt-loaded animals received

third ventricle injections of SDZ SER 082 (160 nmol/rat) 10 min before the central administration of *m*CPP in the same dose. As in the previous experimental set, graduated bottles were reintroduced into the cages 30 min after the last central injection and water intake was recorded for the next 120 min. These groups of animals were compared to an additional group receiving intragastric administration of isotonic saline solution followed by third ventricle injections of saline.

Subcutaneous injections of a 30% PEG solution were used to promote hypovolemia. This solution was subcutaneously administered (2 ml/100 g body weight) 4 h before the intracerebroventricular injections of *m*CPP or isotonic saline solution (controls). Graduated bottles were removed from the cages immediately before PEG administration and reintroduced 30 min after the intracerebroventricular injections. To investigate the participation of 5-$HT_{2B/2C}$ receptors in the effects of third ventricle injections of *m*CPP on water intake during hypovolemia, a group of animals received *m*CPP in a dose of 160 nmol/rat, being pretreated with third ventricle injections of equimolar amounts of SDZ SER 082 10 min before *m*CPP injections. These groups of animals were also compared to an additional group receiving subcutaneous injections of isotonic saline solution in the same volume of PEG administration followed by third ventricle injections of saline. In all cases, in this experimental set, cumulative water intake was measured over the next 90 min.

To investigate if *m*CPP could modify water intake by a nonspecific, general central nervous system inhibition or by a locomotor deficit, we decided to investigate the effect of third ventricle injections of *m*CPP on the intake of a 0.1% saccharin solution, which represents a well-established hedonic behavior in rats (Menani et al. 1998). In this experimental set, after third ventricle cannulations, two different groups of animals kept in the usual individual cages, where the only fluid available was water, were transferred (for 2 h/day, for 7 consecutive days) to a different cage (the test cage) where 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 third ventricle injections of *m*CPP (160 nmol/rat) or saline (controls) 30 min before being transferred to the test cage. The intake of water and saccharin was then recorded for the next 120 min.

2.5. Statistical analysis

We used a computer software package (SigmaStat for Windows, Jandel Scientific, San Rafael, CA) to perform two-way (treatment and time as factors) analysis of variance for repeated measures on each experimental set. Post hoc Student–Newman–Keuls' test was used for comparison of each treatment to its corresponding time in the control groups. With the same software package, we used the Student's *t* test to compare saccharin intake after 120 min between groups treated with *m*CPP or saline. The groups were considered significantly different when $P < .05$.

3. Results

Fig. 1 (Panel A) depicts the effect of third ventricle injections of *m*CPP in different doses on water intake in

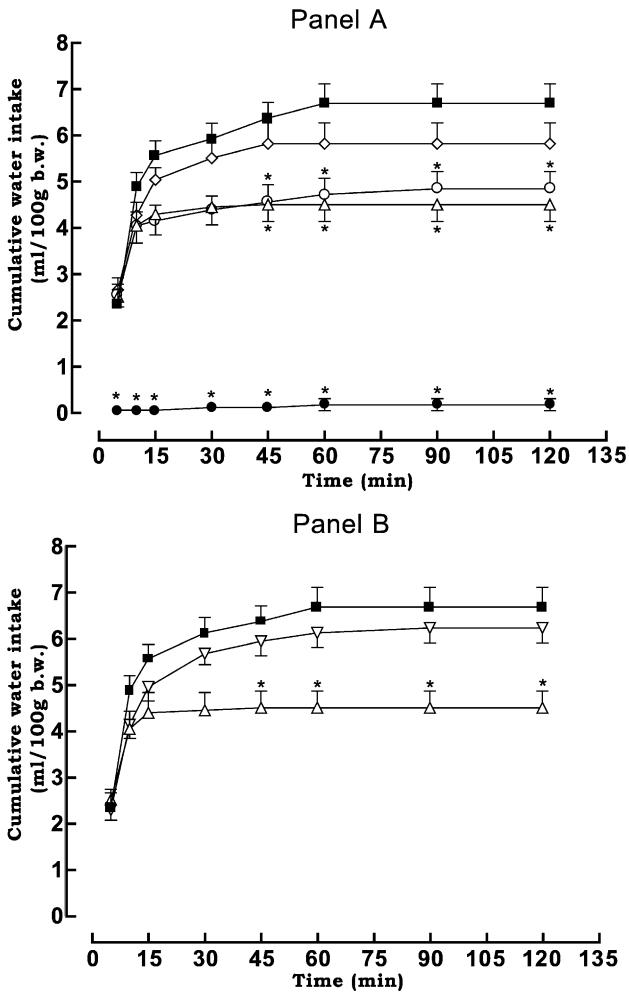

Fig. 1. Cumulative water intake (ml/100 g body weight) of fluid-deprived animals treated with third ventricle injections of *m*CPP, a 5-HT_2 agonist, in several doses (Panel A) and fluid-deprived animals receiving third ventricle injections of *m*CPP but pretreated with SDZ SER 082, a selective 5-$HT_{2B/2C}$ antagonist (Panel B). The following groups are presented in Panel A: saline (■; $n = 14$), *m*CPP 40 nmol/rat (◇; $n = 9$), *m*CPP 80 nmol/rat (○; $n = 12$), *m*CPP 160 nmol/rat (△; $n = 11$). An additional control group of animals not submitted to fluid deprivation and receiving isotonic saline third ventricle injections (normohydrated + saline ●; $n = 10$) is also shown. The following groups are presented in Panel B: saline + saline (■; $n = 12$), saline + *m*CPP 160 nmol/rat (△; $n = 11$), SDZ SER 082 160 nmol/rat + *m*CPP 160 nmol/rat (▽; $n = 12$). Data are presented as mean ± S.E.M. Asterisks indicate a statistically significant difference ($P < .05$) when the distinct groups are compared to fluid-deprived animals receiving saline (Panel A) or fluid-deprived animals pretreated and treated with saline (saline + saline) shown in Panel B. Each curve in the graphs represents data obtained with a naïve group of animals.

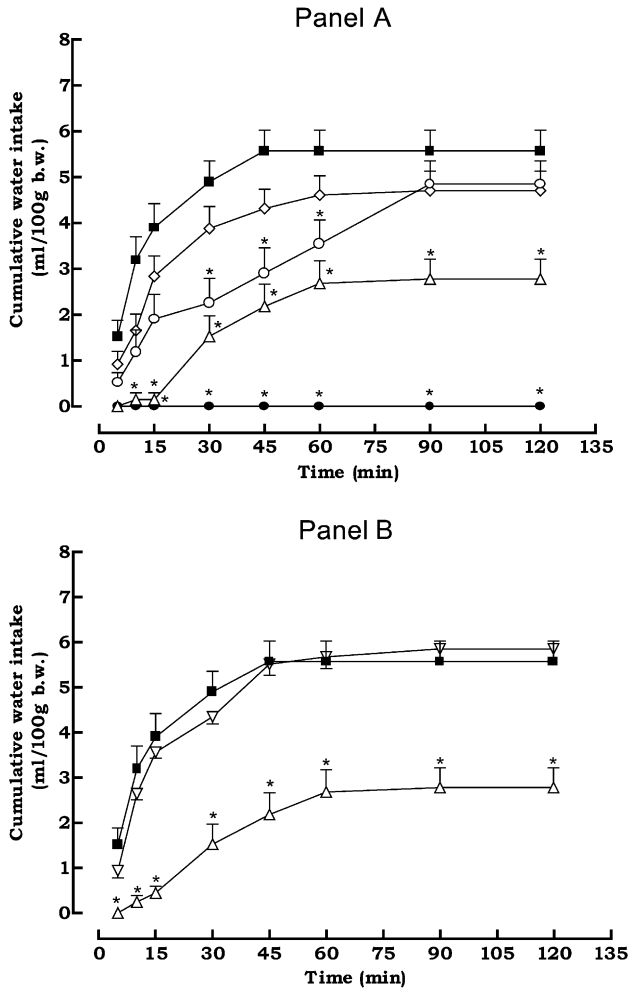

Fig. 2. Cumulative water intake (ml/100 g body weight) of animals receiving an intragastric salt load treated with third ventricle injections of *m*CPP, a 5-HT_2 agonist, in several doses (Panel A) and salt-loaded animals receiving third ventricle injections of *m*CPP but pretreated with SDZ SER 082, a selective 5-$HT_{2B/2C}$ antagonist (Panel B). The following groups are presented in Panel A: saline (■; $n=9$), *m*CPP 40 nmol/rat (✧; $n=9$), *m*CPP 80 nmol/rat (○; $n=9$), *m*CPP 160 nmol/rat (△; $n=10$). An additional control group of animals not submitted to a salt load and receiving isotonic saline third ventricle injections (normal salt + saline ●; $n=10$) is also shown. The following groups are presented in Panel B: saline + saline (■; $n=9$), saline + *m*CPP 160 nmol/rat (△; $n=10$), SDZ SER 082 160 nmol/rat + *m*CPP 160 nmol/rat (▽; $n=13$). Data are presented as mean ± S.E.M. Asterisks indicate a statistically significant difference ($P<.05$) when the distinct groups are compared to fluid-deprived animals receiving saline (Panel A) or fluid-deprived animals pretreated and treated with saline (saline + saline) shown in Panel B. Each curve in the graphs represents data obtained with a naïve group of animals.

fluid-deprived rats. The analysis of variance showed significant main effects for both treatments [$F(4,51)=54.6$, $P=.001$] and time [$F(7,28)=103.2$, $P<.0001$]. As expected, control fluid-deprived animals receiving central injections of isotonic saline present a water intake that is significantly higher than that exhibited by the group of animals not submitted to fluid deprivation also receiving third ventricle injections of isotonic saline. Third ventricle injections of *m*CPP in the lowest dose employed (40 nmol/rat) do not modify water intake in fluid-deprived rats. In the other doses tested (80 and 160 nmol/rat), central *m*CPP administration significantly reduces water intake after 45 min of experiment. Panel B in Fig. 1 shows that central *m*CPP injections (160 nmol/rat) are unable to reduce water intake in fluid-deprived rats pretreated with SDZ SER 082, a selective 5-$HT_{2B/2C}$ antagonist in equimolar amounts.

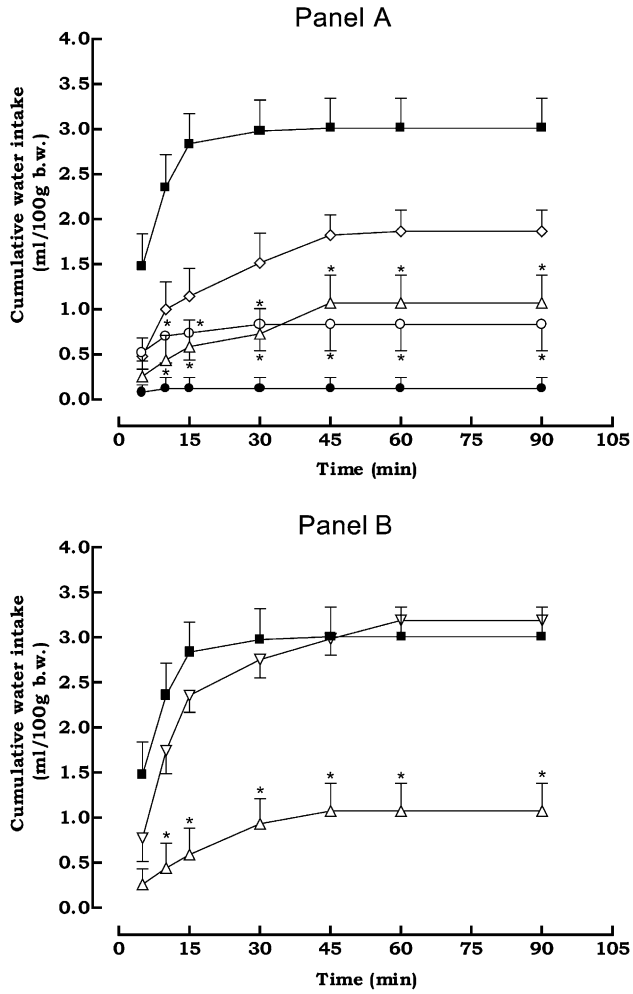

Fig. 3. Cumulative water intake (ml/100 g body weight) of hypovolemic animals treated with third ventricle injections of *m*CPP, a 5-HT_2 agonist, in several doses (Panel A) and hypovolemic animals receiving third ventricle injections of *m*CPP but pretreated with SDZ SER 082, a selective 5-$HT_{2B/2C}$ antagonist (Panel B). The following groups are presented in Panel A: saline (■; $n=12$), *m*CPP 40 nmol/rat (✧; $n=10$), *m*CPP 80 nmol/rat (○; $n=11$), *m*CPP 160 nmol/rat (△; $n=11$). An additional control group of animals not submitted to fluid deprivation and receiving isotonic saline third ventricle injections (euvolemic + saline ●; $n=10$) is also shown. The following groups are presented in Panel B: saline + saline (■; $n=12$), saline + *m*CPP 160 nmol/rat (△; $n=10$), SDZ SER 082 160 nmol/rat + *m*CPP 160 nmol/rat (▽; $n=10$). Data are presented as mean ± S.E.M. Asterisks indicate a statistically significant difference ($P<.05$) when the distinct groups are compared to hypovolemic animals receiving saline (Panel A) or hypovolemic animals pretreated and treated with saline (saline + saline) shown in Panel B. Each curve in the graphs represents data obtained with a naïve group of animals.

Fig. 2 (Panel A) shows the effect of *m*CPP central injections on water intake in rats after an intragastric salt load. The analysis of variance revealed significant differences concerning both treatments [$F(4,41) = 24.47$, $P = .0001$] and time [$F(7,28) = 108.55$, $P < .0001$]. As is predictable, control salt-loaded rats (intragastric hypertonic saline + intracerebroventricular isotonic saline) show a significant enhancement in water intake as compared to rats under a normal salt regimen (intragastric isotonic saline + intracerebroventricular isotonic saline). Central administration of *m*CPP in the lowest dose employed (40 nmol/rat) is unable to modify water intake in rats after a salt load. Third ventricle injections of *m*CPP in the doses of 80 and 160 nmol/rat significantly reduce water intake in rats after a salt load as compared to salt-loaded rats receiving central injections of isotonic saline. However, it is important to note that in the dose of 80 nmol/rat water intake was inhibited within the first hour of the experiment. Panel B shows that the significant reduction in water intake seen in salt-loaded animals receiving central injections of *m*CPP (160 nmol/rat) is not observed in animals pretreated with central injections of SDZ SER 082 in the same dose.

Fig. 3 (Panel A) depicts the effect of central *m*CPP administration on water intake in hypovolemic rats. The analysis of variance yielded significant main effects for both treatments [$F(4,49) = 11.5$, $P < .0001$] and time [$F(6, 24) = 30.06$, $P < .0001$]. Hypovolemic rats receiving third ventricle injections of isotonic saline (hypovolemia + intracerebroventricular saline) display a water intake that is significantly enhanced as compared to normovolemic rats also treated with third ventricle injections of isotonic saline (normovolemia + intracerebroventricular saline). Third ventricle injections of *m*CPP in the lowest dose used (40 nmol/rat) are unable to modify water intake in hypovolemic rats. In the doses of 80 and 160 nmol/rat, the central administration of *m*CPP significantly reduced water intake induced by hypovolemia. As shown in Panel B, pretreatment with third ventricle injections of SDZ SER 082 in the dose of 160 nmol/rat abolishes the reduction in water intake observed after central administration of *m*CPP in the same doses in hypovolemic rats.

Fig. 4 shows that in fluid-deprived animals third ventricle injections of *m*CPP (160 nmol/rat) fail to alter saccharin ingestion after 120 min as compared to control fluid-deprived animals receiving third ventricle injections of isotonic saline.

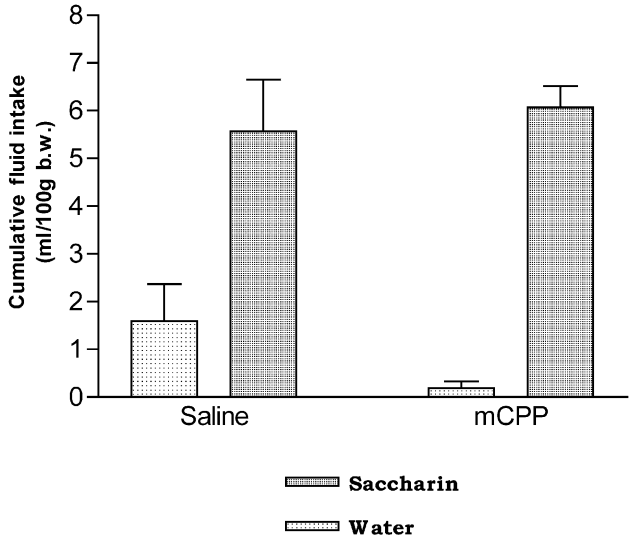

Fig. 4. Cumulative intake of water and 0.1% saccharin solution (ml/100 g body weight) after 120 min in animals submitted to a previous fluid deprivation receiving third ventricle injections of saline (controls) or *m*CPP 160 nmol/rat. Data are expressed as mean ± S.E.M. Each bar in the graph represents data obtained with a naïve group of animals.

4. Discussion

The data presented here show that acute third ventricle injections of *m*CPP, a 5-HT_2 agonist, reduce water intake in three different conditions that represent strong physiological stimuli inducing water intake: fluid deprivation, acute salt load and hypovolemia. This effect appears to depend on the activation of central 5-$HT_{2B/2C}$ receptors, because the pretreatment with SDZ SER 082, a selective 5-$HT_{2B/2C}$ receptor antagonist, abolishes the reduction in water intake induced by central administration of *m*CPP after the different physiological stimuli employed here. The water intake decrease induced by central injections of *m*CPP seems not to be the result of a nonspecific inhibitory behavior or locomotor deficit, because third ventricle injections of *m*CPP do not inhibit saccharin intake, an action that is considered a hedonic behavior in rats (Lieblich et al., 1991).

Previous studies exploring central serotonin pathways and water intake show that electrolytic lesions of the dorsal raphe nucleus, one of the mesencephalic regions containing the cell bodies of brain serotonergic circuitries, induce a significant increase in water intake, indicating that central serotonin may exert an antidipsogenic effect (Tangaprégasson et al., 1973; Tangaprégasson et al., 1974). Also, depletion of brain serotonin contents by *p*-chlorophenylalanine (PCPA) induces the opposite effect (Shisheva et al., 1987). On the other hand, third ventricle injections of nonspecific serotonin agonists like MK 212 inhibit water intake in the rat (Reis et al., 1990). Hindbrain serotonin components seem to have a prominent role in the control of water and salt intake in rats. Indeed, a serotonergic pathway connecting the nucleus of the solitary tract and the area postrema to the lateral parabrachial nucleus (LPBN) seems to exert an inhibitory tonus on water intake, because serotonin antagonists like methylsergide increase water and salt intake when injected into the LPBN (Menani and Johnson, 1995; Menani et al., 1996; Morilak et al., 1993). In addition, serotonin or DOI, a serotonin 5-HT_2 receptor agonist, decreases water intake in rats when injected into the LPBN (Menani and Johnson, 1995). We have previously

demonstrated that third ventricle injections of L-694,247, a 5-HT_{1D} receptor agonist, reduce water intake in rats in several situations (De Castro-e-Silva et al., 1997), while central 5-HT_4 receptors seem to promote a dual effect, potentiating water intake after central angiotensinergic stimulation while inhibiting drinking after central cholinergic activation (Castro et al., 2000). Thus, by interacting with different serotonin receptors, brain serotonin pathways may comprise a complex system capable of influencing the central nervous system modulation of water and salt intake.

Drinking behavior is controlled by complex interactions involving various interconnected brain areas using different neurotransmitters that bind to a multitude of receptors that operate a variety of second messengers. The data presented here seem to consolidate even further the inhibitory role of brain serotonin on water intake. The thirst-inducing physiological stimuli used in each of the experimental protocols employed here activate distinct brain mechanisms. Acute salt loads lead to an increase in plasma osmolarity, a condition that preferentially activates central thirst-triggering cholinergic components, while hypovolemia seems to turn on central angiotensinergic mechanisms promoting water intake (Abdelaal et al., 1976; Block and Fisher, 1975). Central *m*CPP administration was able to decrease water intake both after acute salt loads and hypovolemia, rendering it reasonable to suggest that pharmacological activation of a central 5-HT_2 component efficaciously blunts the thirst-triggering capacity of the physiological stimuli employed here. Both an inhibitory influence exerted by 5-HT_2 receptors on the cholinergic and angiotensinergic pathways linked to the induction of thirst and the stimulation of a 5-HT_2 receptor-dependent brain component that attenuates the thirst-inducing effects of these pathways may help to explain the effects here observed. It is not the objective of the present study to distinguish between these two possibilities. However, it is interesting to note that *m*CPP may reduce acetylcholine release in hippocampal synaptosomes (Bolanos-Jiménez et al., 1994; Harel-Dupas et al., 1991) and that 5-HT_2 receptors are present in GABAergic interneurons that normally inhibit acetylcholine release in many brain areas (Morilak et al., 1993). Thus, it is valid to suggest that acetylcholine inhibition could be a mechanism explaining the reduction in water intake seen in salt-loaded animals treated with *m*CPP.

*m*CPP is a pharmacological agent that preferentially stimulates 5-$HT_{2B/2C}$ receptors (Barnes and Sharp, 1999). In the present study, the effects obtained with the central administration of *m*CPP were reversed by the pretreatment with SDZ SER 082, a selective 5-$HT_{2B/2C}$ receptor antagonist, leading us to suggest that the activation of 5-$HT_{2B/2C}$ receptors in the brain exerts an antidipsogenic effect on the different physiological conditions used here: fluid deprivation, acute salt load and hypovolemia. *m*CPP may display some affinity to 5-HT_{2A} receptors, and an experimental protocol designed to test if 5-HT_{2A} receptor antagonists could modify the effects of *m*CPP observed here is a reasonable idea. However, the drugs able to antagonize 5-HT_{2A} receptors also have prominent effects on nonserotonergic receptors or bind with lesser affinity to 5-HT_{2B} and 5-HT_{2C} receptors. Therefore, their use surely would yield more doubts than certainties. Moreover, the fact that SDZ SER 082 pretreatment does not simply reduce but completely abolishes the effects of *m*CPP observed here favors the interpretation that those effects were specifically dependent on the activation of 5-$HT_{2B/2C}$ receptors.

The 5-HT_{2B} receptor has been recently reported in a few regions of the rat brain (Duxon et al., 1997), while 5-HT_{2C} receptors that seem to exist only in the central nervous system have a much broader localization (Clement et al., 2000). However, both receptors are present in limbic areas that participate in the mechanisms controlling water intake (Barnes and Sharp, 1999; Clement et al., 2000).

Pharmacological tools able to distinguish effects produced by 5-HT_{2B} and 5-HT_{2C} receptors are not yet available. Even some drugs considered as preferential 5-HT_{2C} antagonists still interact with 5-HT_{2B} or other serotonergic receptors (Barnes and Sharp, 1999). Thus, the results obtained here have to be taken, at least for the moment, as the result of central 5-$HT_{2B/2C}$ receptor stimulation.

Peripheral injections of *m*CPP in rats reduce saccharin intake in a two-bottle test as demonstrated by Cooper and Barber (1994), while in the present study saccharin injection was not affected by central *m*CPP administration. The simultaneous activation of peripheral and central serotonergic components by systemic administration of serotonergic agents surely constitutes a model that is rather different from the model we employed here. Quite simply, it is possible that peripheral serotonergic components explain the data obtained by those authors.

It was also demonstrated that peripheral *m*CPP administration reduces salt intake in water-deprived rats (Cooper and Ciccocippo, 1993; Neil and Cooper, 1989b) and that activation of 5-HT_2 receptors located in the lateral parabrachial nucleus seems to decrease water intake without interfering with sucrose intake. These data are well integrated with our results, showing that central 5-$HT_{2B/2C}$ receptors may be part of an operational system that specifically reduces water and salt intake. This system could help to prevent or correct hypervolemia and to maintain blood pressure within its normal range, avoiding hypertension. However, further investigation into the mechanism whereby this possible operational system works is warranted to elucidate the exact roles played by serotonin receptors within and outside the brain in the homeostatic mechanisms controlling body fluid and electrolyte.

Furthermore, it is well known that central *m*CPP administration is able to elicit anoretic effects (Kennet and Curzon, 1988; Samanin et al., 1979). Thus, the water intake inhibition induced by central 5-$HT_{2B/2C}$ receptor activation observed here may indicate the participation of these receptors in a broad component operating ingestive inhibition.

The 5-$HT_{2B/2C}$ agonist *m*CPP was unable to inhibit water intake in the beginning of some experiments. As we injected the drugs into the cerebroventricular system, this may simply indicate that, in the initial period of the sessions, the drug was not yet present in the structures related to water intake control.

The main objective of the present study was to investigate the role of central 5-HT_2 receptors in the regulation of water intake in distinctly different physiological conditions. However, *m*CPP is the main metabolite of the antidepressant trazodone, a substance chemically unrelated to other currently available antidepressants (Haria et al., 1994; Willins and Meltzer, 1997), which displays equal efficacy in the treatment of major depressive episodes when compared to tricyclic antidepressants like desipramine, imipramine and amitriptyline (Rawls, 1982). Furthermore, the systematic use of a wide list of legal and illegal drugs whose target is the brain serotonergic system (serotonin agonists and antagonists, serotonin reuptake inhibitors and serotonin neurotoxic agents like MDMA) renders clinically important the investigation of any aspect of brain serotonin physiology and pharmacology.

The water intake inhibition induced by *m*CPP central injections observed here seems not to represent a general nonspecific central nervous system depression or locomotor deficit. In the present paper, when fluid-deprived animals have the choice to choose between water and a 0.1% saccharin solution, they prefer to drink saccharin. This seems to indicate a hedonic behavior, similar to that of humans preferring to replenish body water using a tasty refreshing beverage instead of water. As this kind of behavior is clearly preserved in animals receiving *m*CPP, it is possible to exclude a general central nervous system depression or a significant locomotor deficit as the cause of the water intake reduction observed in the *m*CPP-treated animals. Moreover, we could not distinguish any difference in the locomotor behavior of animals receiving *m*CPP or saline.

In summary, the present data indicate that third ventricle injections of *m*CPP inhibits water intake due to three different physiological stimuli: fluid deprivation, acute salt load and hypovolemia. This effect seems to depend on the functional integrity of brain 5-$HT_{2B/2C}$ receptors because the central pretreatment with SDZ SER 082, a selective 5-$HT_{2B/2C}$ antagonist, abolishes the reduction in water intake induced by *m*CPP treatment. Water intake inhibition by central *m*CPP administration seems not to be associated to a general nonspecific central nervous system depression or to a locomotor deficit because the ingestion of a saccharin solution is not modified by *m*CPP third ventricle injections.

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