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Central 5-HT_4 Receptors and Drinking Behavior

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CASTRO, L., E. DE CASTRO-E-SILVA, A. K. S. LIMA, F. S. SOUZA, I. MALDONADO, D. F. MACÊDO, M. G. FERREIRA, G. F. SANTAMARIA, I. P. V. BANDEIRA, A. L. M. AMOR, F. L. Q. CARVALHO, M. A. ROCHA, JR., I. R. OLIVEIRA AND J. B. FREGONEZE. *Central 5-HT_4 receptors and drinking behavior*. PHARMACOL BIOCHEM BEHAV **66**(2) 443–448, 2000.—The aim of the present study was to investigate the effect of acute third ventricle injections of two different 5-HT_4 receptor antagonists, GR 113808 and SB 204070, on water intake in different situations. Injections of 80 nmol/rat of both GR 113808 and SB 204070 were unable to modify water intake in normohydrated rats. Pretreatment with GR 113808 (40 and 80 nmol/rat) and SB 204070 (80 and 160 nmol/rat) blunted water intake after third ventricle injections of angiotensin II (9.6 pmol/rat) compared to saline-pretreated controls. Pretreatment with 80 nmol/rat of both antagonists potentiated drinking induced by third ventricle injections of carbachol (11.0 nmol/rat) compared to saline-pretreated control. In all doses employed, none of the compounds was able to modify water intake in dehydrated rats. A separate control test using one-bottle taste aversion paradigm indicated that the reduction in water intake observed in some of the present experiments could not be attributed to a drug-induced malaise. It is suggested that central 5-HT_4 receptors exert a dualistic role on the control of water intake potentiating angiotensin II-induced drinking and inhibiting thirst induced by central cholinergic activation

5-HT_4 receptors Drinking behavior Third ventricle injections

BRAIN serotonin's ubiquitous distribution, its widespread interactions with other neurotransmitters, and the availability of a large family of distinct receptors render favorable a multitude of central serotoninergic actions (21). Serotonin-induced satiety has been extensively studied and reviewed (30). In contrast, central serotonin influences on drinking behavior have been receiving very little attention. A general review of the literature indicates that central serotonin may exert an inhibitory action on water intake in rats, although some studies found contradictory results (23,28). As far as we know, a systematic screening of serotonin receptor subtypes involved in this response is still lacking. We have previously demonstrated that postsynaptic 5-HT_{1D} receptors in the vicinities of the third ventricle seem to exert a negative effect on water intake in several conditions, such as with dehydration and after pharmacological induction of drinking by central administration of cholinergic, β-adrenergic and angiotensinergic agents (5).

First described in mouse colliculi neurons (6), central 5-HT_4 receptors exhibit a widespread localization in the mammalian brain. They are especially prominent in the limbic structures as well as in other neuronal circuitries like the cortico–striatal–tectal and the septo–hippocampal–habenulo–interpeduncular pathways (8). Previous reports indicate central 5-HT_4 receptor involvement in cognitive processing (2), modulation of dopamine and acetylcholine release, anxiety (8), and memory (16).

A complex interactive network of inhibitory and stimulatory inputs, involving different brain neurotransmitters and areas, mediates thirst generation. Central cholinergic, angiotensinergic, and β-adrenergic pathways seem to induce water intake, while α-adrenergic, opioidergic, and serotonergic circuitries exert the opposite effect (14).

In the present article, using a pharmacological approach, we investigated the possible role of central 5-HT_4 receptors in

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the control of water intake in normohydrated rats after cholinergic and angiotensinergic stimulation as well as in dehydrated rats.

METHOD

We used adult male Wistar rats (230 ± 20 g) kept under controlled light (lights on from 0500 to 1900 h) and temperature (24 ± 2°C) conditions. They had free access to tap water and laboratory chow (Nuvital Nutrientes Ltda., Curitiba, Brazil).

Surgical Procedure

The third ventricle was cannulated under sodium pentobarbital anesthesia (50 mg/kg IP) as described elsewhere (1). In brief, after positioning in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA), a chronic 28 gauge guide cannula was implanted according to the following coordinates: anteroposterior = 0.5 mm behind the bregma; lateral = 0.0 mm; vertical = 8.0 mm below the skull. The animals were fixed to the stereotaxic with the head inclined slightly upward, avoiding lesions to the subfornical organ by the cannula. Two screws fixed to the skull bone with dental acrylic held the cannula. After surgery, the animals were housed in individual cages for 7 days before the experiments.

Drugs and Microinjections

The following drugs were used: carbachol, (Asp^1-Ileu^5-AII) angiotensin II (AII) and lithium chloride were acquired from Sigma Chemical Co., St. Louis, MO. SB 204070 (8-amino-7-chloro-(N-butyl-piperidy-methylbenzene-1,4-dioxan-5-carboxylate hydrochloride), a specific 5-HT$_4$ receptor antagonist, was generously donated by SmithKline Beecham Pharmaceuticals, UK. GR 113808 [1-[2-(methylsulphonylamino)ethyl]-4-piperidinyl]methyl 1-methyl-1H-indole-3-carboxylate), a second 5-HT$_4$ receptor antagonist, was a generous gift from GlaxoWellcome Research and Development Limited, UK.

All drugs were injected into the third ventricle in a volume of 2 μl in a period of 90 s. They were always dissolved in isotonic saline solution. Third ventricle injections were accomplished using Hamilton microsyringes connected to a 28-gauge injection cannula by a polyethylene tubing extension (PE10). Immediately after sacrifice, a small amount of blue Evans dye was injected into the cannulas. After brain removal and slicing, careful examination was undertaken to verify the position of the cannulas in the brain. We have taken into consideration only those animals whose cannulas were correctly placed into the third ventricle.

Experimental Design

A taste aversion test was performed to ascertain that both 5-HT$_4$ antagonists were devoid of nonspecific inhibitory "illness-like" effects on water intake. We employed the following protocol based on the experimental design proposed by Nachman (22), in which lithium chloride is used to make rats ill in temporal association with the novel taste of saccharin: 7 days after surgery, all animals had their access to water restricted to 15 min/day (between 1200 and 1215 h) during 4 consecutive days. On the fifth day, the animals were divided into four different groups. The first group (controls) received two immediately consecutive injections of isotonic saline solution—the first being intraperitoneal (0.6% b.wt.), and the second into the third ventricle (2 μl). The second group received intraperitoneal injections of lithium chloride 0.15 M (0.6% b.wt.) followed by third ventricle injections of isotonic saline solution. The third group received intraperitoneal injections of isotonic saline solution, in the same amount used in the previous group, followed by third ventricle injections of GR 113808 (80 nmol/rat). The fourth group received intraperitoneal injections of isotonic saline solution, in the same amount used in the previous group, followed by third ventricle injections of SB 204070 (80 nmol/rat). On this same day, all groups of animals had access to bottles containing saccharin (0.25%) for 1 min, immediately before the injections, and for an additional 14 min immediately after. On the next day, at the same time that bottles had been available on the previous days (1200 to 1215 h), saccharin-containing bottles were introduced into all cages and the volume ingested recorded.

To investigate central 5-HT$_4$ receptor participation in water intake control in normohydrated animals, both 5-HT$_4$ antagonists (SB 204070 and GR 113808) were injected into the third ventricle (80 nmol/rat), and the cumulative water intake was recorded for the next 120 min. To study the effect of central 5-HT$_4$ receptors on AII-induced water intake in normohydrated animals, both antagonists were centrally injected in several doses (GR 113808, 20, 40, and 80 nmol/rat; SB 204070, 40, 80, and 160 nmol/rat) 30 min before third ventricle injections of AII (9.6 pmol/rat), and the cumulative water intake was recorded for the next 120 min. To evaluate the participation of central 5-HT$_4$ receptors on cholinergic-stimulated drinking in normohydrated animals both antagonists were injected into the third ventricle, in the same doses referred to before, 30 min before receiving central injections of carbachol (11 nmol/rat). Then, the cumulative water intake was recorded for the next 120 min. To study the participation of central 5-HT$_4$ receptors in water intake after dehydration, two distinct groups of rats underwent water deprivation for 14 h immediately before the experimental session. The first group received third ventricle injections of GR 113808 (80 and 160 nmol/rat), and the second received SB 204070 (80 and 160 nmol/rat) by the same route. Graduated bottles were reintroduced into the cages 30 min after the administration of both compounds, and water intake recorded for the next 120 min.

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. A post hoc Student–Newman–Keuls test was used for comparison of each treatment to its corresponding time in the control groups. The groups were considered significantly different when $p < 0.05$. The data are presented as mean ± SEM. The Student's *t*-test was used to analyze the data concerning the aversion test.

RESULTS

Figure 1 shows the result of the aversion test performed to ascertain whether any of the 5-HT$_4$ antagonists were able to induce "illness-like" side effects. As expected, animals making a previous association between lithium chloride and saccharin display a significant reduction in saccharin intake on the next day compared to saline-treated controls. In contrast, the previous association of each of the antagonists with saccharin failed to produce any decrease in saccharin intake the next day, indicating that both compounds are devoid of illness-inducing effects.

Figure 2 reveals the effect of central pretreatment with GR 113808 on AII-induced water intake in normohydrated

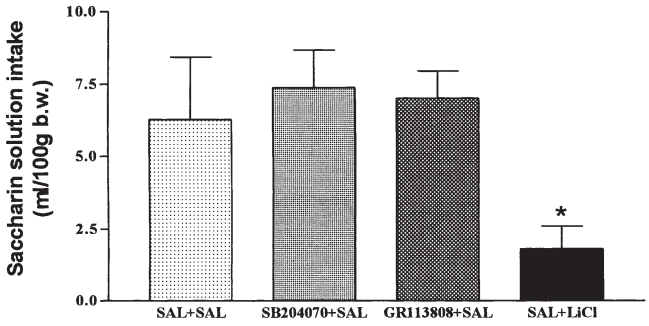

FIG. 1. Saccharin solution (0.25%) consumption (ml/100 g body weight) over 15 min at a second offering. Under each bar in the graph is indicated the sequence of injections used during the first saccharin offering, the first being into the third ventricle, and the second via intraperitoneal route. Data are expressed as mean ± SEM. The asterisk indicates a statistically significant difference ($p < 0.01$) between that particular group and controls (sal + sal).

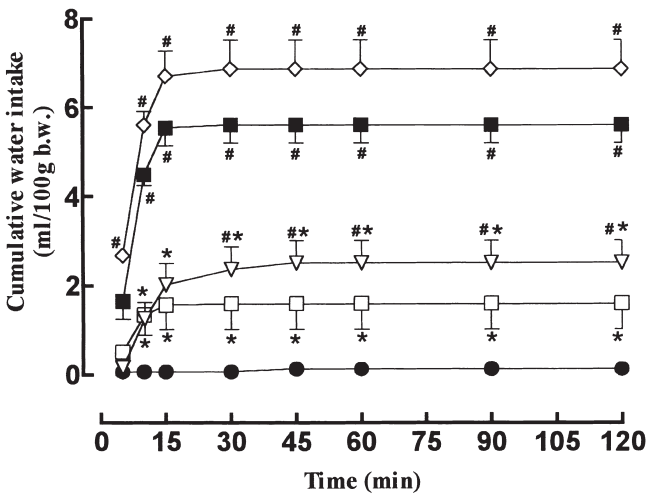

FIG. 2. Cumulative water intake (ml/100 g body weight) in normohydrated rats receiving third ventricle injections of saline (controls) or GR 113808 in several doses 30 min before third ventricle administration of AII (9.6 pmol/rat). The following groups were formed: sal + sal (●; $n = 12$); sal + AII (■; $n = 11$); GR 113808 20 nmol/rat + AII (◇; $n = 9$); GR 113808 40 nmol/rat + AII (▽; $n = 11$); GR 113808 80 nmol/rat + AII (□; $n = 11$). Data are presented as mean ± SEM. ANOVA test: treatments, $F(4, 49) = 42.4$, $p < 0.0001$; time, $F(7, 28) = 102.5$, $p < 0.0001$. Asterisks indicate a statistically significant difference (Student–Newman–Keuls test; $p < 0.05$) compared to the sal + AII group. # Indicate a statistically significant difference (Student–Newman–Keuls test; $p < 0.05$) compared with the sal + sal group.

animals. The ANOVA yielded significant main effects for both treatments, $F(4, 49) = 42.4$, $p < 0.0001$, and time, $F(7, 28) = 102.5$, $p < 0.0001$. Animals receiving third ventricle injections of 9.6 pmol of AII and pretreated with saline (saline + AII) exhibit a significant increase in water intake when compared to the control group pretreated and treated with saline (saline + saline). Pretreatment with GR 113808 in the smallest dose employed (20 nmol/rat) did not modify AII-induced water intake. The groups pretreated with GR 113808 in doses of 40 and 80 nmol/rat (GR 113808 + AII) show a significant blockade in water intake induced by third ventricle AII injections.

Figure 3 displays the effect of central pretreatment with SB 204070 on water intake induced by third ventricle injections of AII (9.6 pmol/rat). The ANOVA test showed significant differences among treatments, $F(4, 28) = 24.8$, $p < 0.0001$, and time, $F(7, 28) = 73.3$, $p < 0.0001$. As in the previous experimental set, the saline + AII group exhibits a significantly higher water intake compared to controls (saline + saline). In the smallest dose employed (40 nmol/rat) SB 204070 did not alter AII-induced thirst. However, groups receiving SB 204070 in the doses of 80 and 160 nmol/rat (SB 204070 + AII) present a significant decline in AII-induced water intake.

Figure 4 expresses the effects of pretreatment with GR 113808 on water intake induced by third ventricle carbachol injections [ANOVA test: treatment, $F(3, 34) = 37.1$, $p < 0.0001$; time, $F(7, 21) = 190.8$, $p < 0.0001$]. Animals receiving central injections of carbachol (11.0 nmol/rat) pretreated with saline (saline + carbachol) drank significantly more water than did controls (saline + saline). Animals receiving carbachol but pretreated with GR 113808 (GR 113808 + carbachol) in the highest dose employed (80 nmol/rat) exhibit a dipsogenic response that is significantly greater than that displayed by animals receiving carbachol but pretreated with saline (saline + carbachol).

Figure 5 describes the effect of pretreatment with SB 204070 on carbachol-induced water intake [ANOVA test: treatment, $F(3, 38) = 55.5$, $p < 0.0001$; time, $F(7, 21) = 282.1$, $p < 0.0001$]. Again, the saline + carbachol group presents a significant increase in water intake compared to controls (saline + saline). Pretreatment with SB 204070 in the lowest dose employed (40 nmol/rat) did not modify carbachol-induced drinking. After 60 min, the group pretreated with SB 204070 in the highest dose of 80 nmol/rat (SB 204070 + carbachol) drinks significantly more water when compared to the saline + carbachol group.

Table 1 condenses water intake of normohydrated and dehydrated rats over the next 120 min after reintroduction of the graduated bottles into the cages, in animals treated with third ventricle injections of GR 113808, SB 204070, or saline (controls). No statistically significant difference was found among these groups.

DISCUSSION

The data presented here demonstrate that both 5-HT$_4$ antagonists used (GR 113808 and SB 204070) are devoid of "sickness-like" effects able to impair water intake. It is also clear that both antagonists blunt AII-induced drinking and potentiate carbachol-induced thirst. This suggests that central 5-HT$_4$ receptors may exert a positive drive on AII-induced thirst and an opposite effect on cholinergic-stimulated drinking. None of the compounds modified water intake in normohydrated animals whose drinking was not stimulated by central injections of dipsogenic agents. Additionally, both compounds failed to change water intake in dehydrated animals.

The few studies in the literature exploring the role of brain serotonin in the control of water intake are far from conclusive. However, taking those studies into consideration, most evidence favors central serotoninergic inhibition of water intake. Thus, electrolytic lesions of the dorsal raphe nucleus promote a remarkable increase in water intake (32,33) and *p*-chlorophenylalanine, a neurotoxic agent that depletes brain serotonin content, induces a significant dipsogenic action

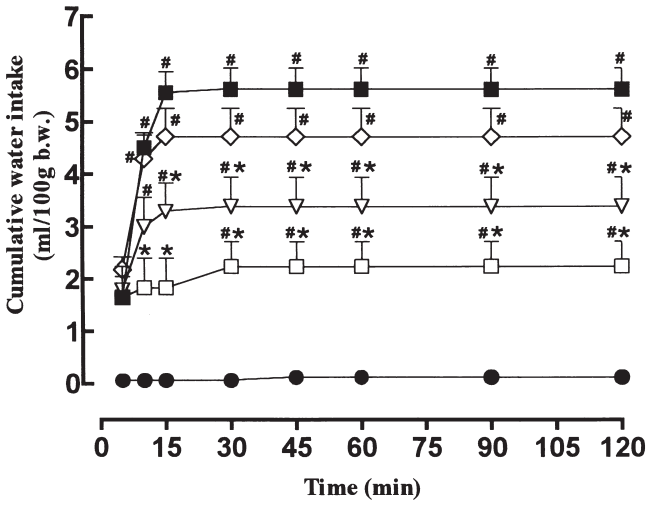

FIG. 3. Cumulative water intake (ml/100 g body weight) in normohydrated rats receiving third ventricle injections of saline (controls) or SB 204070 in several doses 30 min before third ventricle administration of AII (9.6 pmol/rat). The following groups were formed: sal + sal (●; $n = 12$); sal + AII (■; $n = 11$); SB 204070 40 nmol/rat + AII (◇; $n = 11$); SB 204070 80 nmol/rat + AII (▽; $n = 11$); SB 204070 160 nmol/rat + AII (□; $n = 8$). Data are presented as mean ± SEM. ANOVA test: treatments, $F(4, 28) = 24.8$, $p < 0.0001$; time, $F(7, 28) = 73.3$, $p < 0.0001$. Asterisks indicate a statistically significant difference ($p < 0.05$) compared to the sal + AII group. #Indicates a statistically significant difference ($p < 0.05$) compared with the sal + sal group.

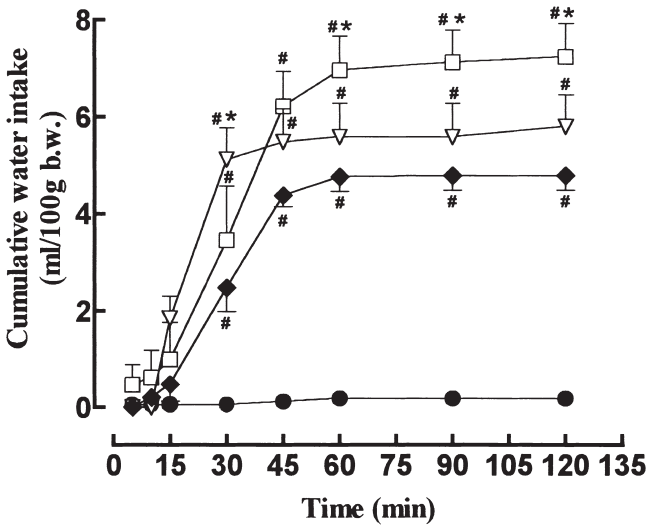

FIG. 4. Cumulative water intake (ml/100 g body weight) in normohydrated rats receiving third ventricle injections of saline (controls) or GR 113808 in several doses 30 min before third ventricle administration of carbachol (11 nmol/rat). The following groups were formed: sal + sal (●; $n = 12$); sal + carbachol (◆; $n = 11$); GR 113808 40 nmol/rat + carbachol (▽; $n = 8$); GR 113808 80 nmol/rat + carbachol (□; $n = 7$). Data are presented as mean ± SEM. ANOVA test: treatments, $F(3, 34) = 37.1$, $p < 0.0001$); time, $F(7, 21) = 190.8$, $p < 0.0001$. Asterisks indicate a statistically significant difference ($p < 0.05$) compared to the sal + carbachol group. #Indicate a statistically significant difference ($p < 0.05$) compared with the sal + sal group.

(28). In the same direction, third ventricle injections of MK-212, a serotonergic 5-HT_2/5-HT_{1C} agonist, significantly reduce water intake in the rat (24). Interestingly, when administered by peripheral routes, serotonin and serotonin agonists seem to induce an increase in water intake probably as a consequence of peripheral AII release (25,29). The fact that central serotonin induces an inhibitory effect on water intake was also established by our laboratory. Indeed, we recently demonstrated that activation of central postsynaptic 5-HT_{1D} receptors located in regions around the third ventricle leads to a significant decrease in water intake both in dehydrated rats and in those whose drinking was stimulated by central injections of AII, carbachol and isoproterenol (5).

The 5-HT_4 receptor antagonists used here did not modify water intake in normohydrated animals whose drinking was not stimulated by central pharmacological angiotensinergic or cholinergic activation. This may simply mean that under normohydration central 5-HT_4 receptors do not play any important role in the control of water intake. On the other hand, the same compounds were able to diminish AII-induced water intake. This clearly suggests that central 5-HT_4 receptors may exhibit an endogenous potentiating tone favoring drinking behavior after angiotensinergic stimulation. Immediately after central AII administration rats start drinking. This means that AII triggers the motivational input that induces the animal to seek and to drink water. It seems that 5-HT_4 receptors exert a facilitatory drive at some point between angiotensinergic neurons and the higher integrative areas commanding the motor events necessary to water seeking and drinking behavior.

The central blockade of 5-HT_4 receptors by both antagonists (GR 113808 and SB 204070) potentiated carbachol-induced water intake. This indicates that central 5-HT_4 receptors may exert an inhibitory influence on cholinergic-induced drinking. In a manner that is opposite to their action on AII-induced drinking, 5-HT_4 receptors seem to block some crucial step that links cholinergic activation to the generation of the required motivational state leading to water seeking and drinking. Taken together, these data suggest a dualistic role for central 5-HT_4 receptors in the control of water intake: potentiation of AII dispsogenic effect and inhibition of cholinergic-induced drinking.

Redundant brain mechanisms control water intake. Central angiotensinergic and cholinergic circuitries operate parallel systems supporting body fluid homeostasis. Hypovolemia correction, a situation that requires water and sodium intake, is mainly mediated by AII, while the homeostatic responses correcting osmotic dehydration, when salt intake is not required, are chiefly carried out by cholinergic pathways. The blockade of both systems is necessary to impair water intake after dehydration [see (26) for review]. Thus, the dualistic action of 5-HT_4 receptors may explain the absence of effect of both 5-HT_4 receptor antagonists on water intake in dehydrated animals: the potentiation by 5-HT_4 receptors in angiotensinergic thirst-triggering areas being compensated by the inhibition of cholinergic-induced drinking.

It has recently been demonstrated that several hypothalamic nuclei contain 5-HT_4 receptor mRNA (35). Thus, the effects here observed may represent the actions of serotonin interneurons containing 5-HT_4 receptors located in regions in the vicinity of the third ventricle associated with important microregulatory circuitries designed to achieve fine adjustment of neurovegetative and behavioral parameters.

Serotonin modulation of water intake is probably very complex, and may represent a link between hindbrain and forebrain areas involved in thirst control. Immunohistochemi-

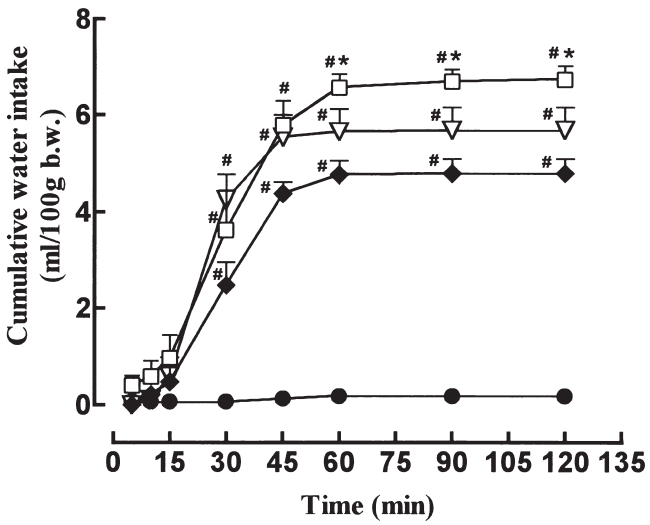

FIG. 5. Cumulative water intake (ml/100 g body weight) in normohydrated rats receiving third ventricle injections of saline (controls) or SB 204070 in several doses 30 min before third ventricle administration of carbachol (11 nmol/rat). The following groups were formed: sal + sal (●; $n = 12$); sal + carbachol (◆; $n = 11$); SB 204070 40 nmol/rat + carbachol (▽; $n = 10$); SB 204070 80 nmol/rat + carbachol (□; $n = 9$). Data are presented as mean ± SEM. ANOVA test: treatments, $F(3, 38) = 55.5$, $p < 0.0001$); time, $F(7, 21) = 282.1$, $p < 0.0001$. Asterisks indicate a statistically significant difference ($p < 0.05$) compared to the sal + carbachol group. #Indicate a statistically significant difference ($p < 0.05$) compared with the sal + sal group.

cal studies reveal that the SFO receives profuse afferent projections coming from the midbrain raphe nuclei (17,18). As stated elsewhere (14), a mass of information generated by baroreceptors, cardiopulmonary, and visceral receptors reaching the midbrain makes serotonin released from the area postrema/nucleus of solitary tract (NTS) complex influence forebrain thirst-controlling areas via lateral parabrachial nuclei (LPBN). Indeed, serotonin 5-HT$_{2A}$/5-HT$_{2C}$ agonists directly injected into the LPBN reduced water intake after central angiotensinergic stimulation (20). Also, serotoninergic mechanisms associated with the LPBN inhibit AII-induced salt intake (4,19). Taking into account the data available in the literature and those presented here, it is reasonable to suggest that serotonin coming from hindbrain structures besides acting on forebrain 5-HT$_{2A}$/5-HT$_{2C}$ and 5-HT$_{1D}$ receptors may also modulate drinking by its dualistic action on 5-HT$_4$ receptors.

TABLE 1

AVERAGE (±SEM) CUMULATIVE WATER INTAKE (ml/100 g) OF NORMOHYDRATED AND DEHYDRATED RATS UNDER DIFFERENT TREATMENTS

Animal condition/Treatment	Normohydrated	Dehydrated
Isotonic saline	0.18 ± 0.13 ($n = 12$)	7.13 ± 0.22 ($n = 11$)
GR113808 (80 nmol)	0.09 ± 0.07 ($n = 13$)	6.16 ± 0.67 ($n = 8$)
GR113808 (160 nmol)	—	7.48 ± 0.47 ($n = 10$)
SB204070 (80 nmol)	0.07 ± 0.05 ($n = 11$)	5.74 ± 0.53 ($n = 10$)
SB204070 (160 nmol)	—	5.56 ± 0.39 ($n = 7$)

The number of animals used in each experiment is indicated within parentheses. Saline, GR 113808, or SB 204070 were injected 30 min before the reintroduction of graduated bottles into the cages. The values express water intake monitored by the next consecutive 120 min.

In opposition to the articles previously quoted asserting central serotonin inhibition of thirst, it is also known that injections of serotonin directly into the SFO may enhance drinking in rats (31), and central injections of serotonin in monkeys may induce water intake (9). Also, depending on the receptor stimulated, serotonin may generate excitatory or inhibitory responses on SFO neurons (27). The dualistic role played by central 5-HT$_4$ receptors here evidenced constitutes additional information explaining the fact that, depending on the experimental protocol used, central serotonin stimulation yields positive or negative effects on water intake.

Serotonin 5-HT$_4$ receptor function in the brain is a rather underexplored matter, because molecular cloning of rat 5-HT$_4$ receptors is very recent (3,13). Indeed, few data link this receptor subtype to specific functions. Among other things, this represents the scarcity of specific pharmacological tools. The 5-HT$_4$ receptor antagonists used here, GR 113808 (10) and SB 204070 (11), seem to be highly selective. As both compounds generated the same pattern of responses in each of the experimental sets, we believe that our data are the result of specific 5-HT$_4$ receptor blockade, and do not represent spurious pharmacological properties unrelated to serotoninergic mechanisms. In the present experimental protocol, we selectively blocked 5-HT$_4$ receptors and inferred that the effect observed represents the opposite of what would be obtained after 5-HT$_4$ receptor stimulation. It would be tempting to use direct pharmacological activation of 5-HT$_4$ receptors through the use of selective agonists. Unfortunately, no such pharmacological tool is yet available. In fact, most 5-HT$_4$ receptor agonists are rather nonselective or behave as partial agonists.

There is a large potential for the therapeutic use of 5-HT$_4$ receptor agonists and antagonists in the near future (12). Also, some classical drugs like metoclopramide (an antiemetic and gastroprokinetic agent included in several therapeutic routines) are nonselective 5-HT$_4$ receptor agonists that easily cross the blood–brain barrier, reaching central 5-HT$_4$ receptors (7). Furthermore, 5-HT$_4$ receptor antagonists are being indicated as therapeutic agents in urinary incontinence (15), because 5-HT$_4$ receptors facilitate the contraction of dextrusor muscle (34). Thus, the investigation of functional aspects of central 5-HT$_4$ receptors, besides filling a gap in the knowledge of basic neuroscience, may be relevant in clinical pharmacology.

In summary, the data presented here indicate that central 5-HT$_4$ receptors exert a dualistic role on the control of water intake potentiating AII-induced drinking and inhibiting thirst induced by central cholinergic activation.

ACKNOWLEDGEMENTS

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