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Effect of the intracerebroventricular administration of GR 113808, a selective 5-HT_4 antagonist, on water intake during hyperosmolarity and hypovolemia

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Abstract

We demonstrate here that acute third ventricle injections of GR 113808, a highly selective 5-HT_4 antagonist, decrease water intake induced by a previous salt load while potentiating drinking elicited by hypovolemia induced by previous subcutaneous administration of polyethylene glycol in male Wistar rats (200 ± 20 g). At the dose of 160 nmol/rat, third ventricle injections of GR 113808 induced a significant reduction of water intake in salt-loaded animals after 120 min as compared to salt-loaded animals receiving third ventricle injections of saline (salt load + GR = 3.44 ± 0.41 ml, N = 12; salt load + saline = 5.74 ± 0.40 ml, N = 9). At the dose of 80 nmol/rat, GR 113808 significantly enhanced water intake in hypovolemic animals after 120 min as compared to hypovolemic animals receiving third ventricle injections of saline (hypovol + GR = 4.01 ± 0.27 ml, N = 8; hypovol + saline = 2.41 ± 0.23 ml, N = 12). We suggest that central 5-HT_4 receptors may exert a positive drive on water intake due to hyperosmolarity and a negative input on drinking provoked by hypovolemia.

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Distinct patterns of serotonin receptor subtype distribution in the central nervous system seem to be essential for the expression of multiple serotonin effects mediated by a large number of G protein-coupled receptors (1,2). In spite of a large amount of recent work, a comprehensive picture of the physiological roles played by central serotonin receptors is needed. In particular, the possible functions exerted by central 5-HT_4 receptors are only partially known. Displaying high densities in several regions of the brain, such as the olfactory tubercle, nucleus accumbens, ventral pallidum, septal region, hippocampus and amygdala (3,4), central 5-HT_4 receptors may be linked to processes such as anxiety, memory and cognition (5).

Central serotonin involvement in the control of water intake has not been fully established. While some studies indicate that brain serotonin exerts a negative effect on drinking behavior (6,7), others suggest the opposite

(8,9). We have recently demonstrated that central 5-HT_4 receptors may modulate water intake in rats, potentiating drinking behavior induced by angiotensin II injections, while reducing water intake provoked by central cholinergic stimulation (6).

In the present study, we investigated the role of central 5-HT_4 receptors in the control of water intake in rats in two different conditions: hyperosmolarity due to an acute intragastric salt load, and hypovolemia promoted by subcutaneous administration of polyethylene glycol (PEG).

We used adult male Wistar rats (220 ± 20 g) kept under controlled light (lights on from 5:00 to 19:00 h) and temperature (24 ± 2°C) conditions. The animals had free access to tap water and laboratory chow (Nuvital Nutrientes Ltda., Curitiba, PR, Brazil). Under sodium pentobarbital anesthesia (40 mg/kg, *ip*) the animals were placed in a stereotaxic frame and a chronic cannula was implanted into the third ventricle according to the following coordinates: anteroposterior 0.5 mm behind the bregma, lateral 0 mm, and vertical 8.0 mm below the skull. Two screws fixed to the skull were embedded in dental acrylic and held the cannula. After surgery the rats were housed in individual cages for 7 days before the experiments. The following drugs were used: GR 113808 ([1-[2-(methylsulphonylamino)ethyl]-4-piperidinyl]methyl 1-methyl-1H-indole-3-carboxylate), a 5-HT_4 receptor antagonist, was a generous gift from GlaxoWellcome Research and Development Limited, Hertfordshire, UK; PEG (MW 15,000-20,000) was purchased from Sigma Chemical Co., St. Louis, MO, USA. The drugs were dissolved in saline solution. Third ventricle injections were performed using a Hamilton microsyringe connected to a Mizzy-Slide-Pak needle by a polyethylene extension (PE 10). A total volume of 2 µl was injected over a period of 60 s. After the experiments, a 0.5-µl amount of 0.5% Evans blue dye was injected through the cannula. Following sacrifice and brain removal, the position of the cannula was confirmed by macroscopic analysis of the brain regions stained with Evans blue. Only data from animals whose cannulas were correctly placed into the third ventricle were taken into consideration. To induce a salt load, animals were fasted for 14 h, from 18:00 to 8:00 h the night before the experiment. Ten minutes after the third ventricle injection of a given dose of GR 113808 or isotonic saline solution (controls) the animals received 1 ml/100 g of 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. These groups of animals were compared to an additional group receiving an intragastric administration of isotonic saline solution followed by third ventricle injections of saline.

To induce hypovolemia, a 30% PEG solution was administered subcutaneously (2 ml/100 g) 4 h before the *icv* injections of GR 113808 or isotonic saline solution (controls). Graduated bottles were removed from the cages immediately before PEG administration and reintroduced 30 min after the *icv* injections. Cumulative water intake was measured over the next 120 min. These groups of animals were also compared to an additional group receiving subcutaneous injections of isotonic saline solution in the same volume as used for PEG administration followed by third ventricle injections of saline.

A computer software package (SigmaStat for Windows, Jandel Scientific, San Rafael, CA, USA) that performs two-way (treatment and time as factors) analysis of variance for repeated measures on each experimental set was used. The *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 reported as means ± SEM.

Figure 1 shows the effect of plasma hyperosmolarity induced by an intragastric salt load on water intake in rats receiving third ventricle injections of GR 113808 or isotonic saline solution. As expected, animals receiving an intragastric salt load plus third ventricle injections of isotonic saline (salt load + saline) displayed a significant increase in water intake as compared to rats receiving an intragastric administration of isotonic saline solution plus third ventricle injections of isotonic saline (normal salt + saline). Third ventricle injections of GR 113808 at the dose of 40 nmol/rat (salt load + GR 40 nmol) were unable to modify the high water intake induced by the previous salt load. At the dose of 80 nmol/rat (salt load + GR 80 nmol) GR 113808 induced a significant inhibition of water intake beginning after 30 min and lasting until the end of the experiment. At the dose of 160 nmol/rat (salt load + GR 160 nmol) GR 113808 produced a significant reduction in water intake that lasted for the entire duration of the experiment.

Figure 2 shows the effects of third ventricle injections of GR 113808 at different doses on water intake in hypovolemic animals. Drinking behavior in hypovolemic animals receiving third ventricle injections of isotonic saline (hypovol + saline) was significantly increased compared to normovolemic controls also receiving third ventricle injections of saline (normovol + saline). At the dose of 20 nmol/rat (hypovol + GR 20 nmol) GR 113808 was unable to modify the high water intake induced by hypovolemia. However, in hypovolemic rats, GR 113808 at the doses of 40 nmol/rat (hypovol + GR 40 nmol) and 80 nmol/rat (hypovol + GR 80 nmol) significantly potentiated water intake during the entire duration of the experiment.

The data obtained in the present study clearly show that the central blockade of 5-HT$_4$ receptors by third ventricle injections of GR 113808, a highly selective 5-HT$_4$ an-

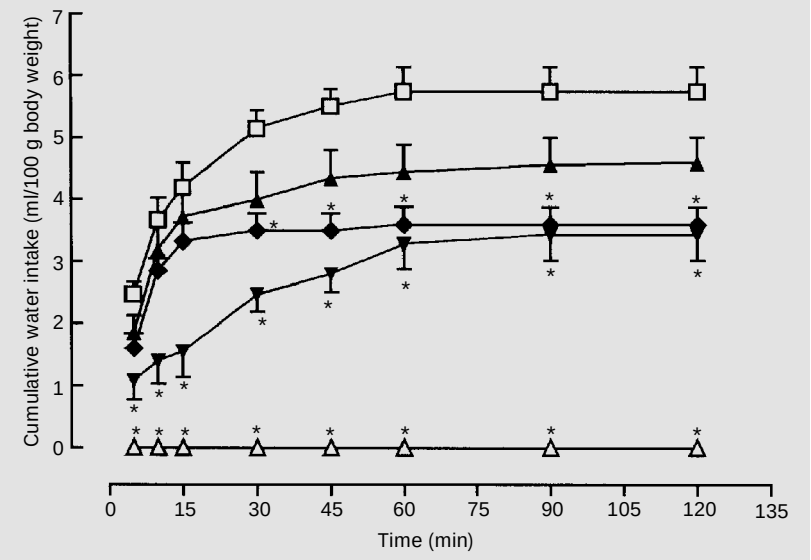

Figure 1. Cumulative water intake (ml/100 g body weight) in animals submitted to a previous intragastric salt load receiving third ventricle injections of GR 113808 at different doses or isotonic saline solution (controls). The following groups are represented: salt load + saline (squares; N = 9); salt load + GR 40 nmol/rat (filled triangles; N = 13); salt load + GR 80 nmol/rat (filled lozenges; N = 9); salt load + GR 160 nmol/rat (inverted filled triangles; N = 12). An additional group receiving intragastric isotonic saline solution under the same conditions as used to promote salt load and receiving isotonic saline injections in the third ventricle (normal salt + saline, open triangles; N = 13) is also shown. Data are reported as means ± SEM. *P<0.05 compared to salt load + saline (two-way analysis of variance followed by the Student-Newman-Keuls test).

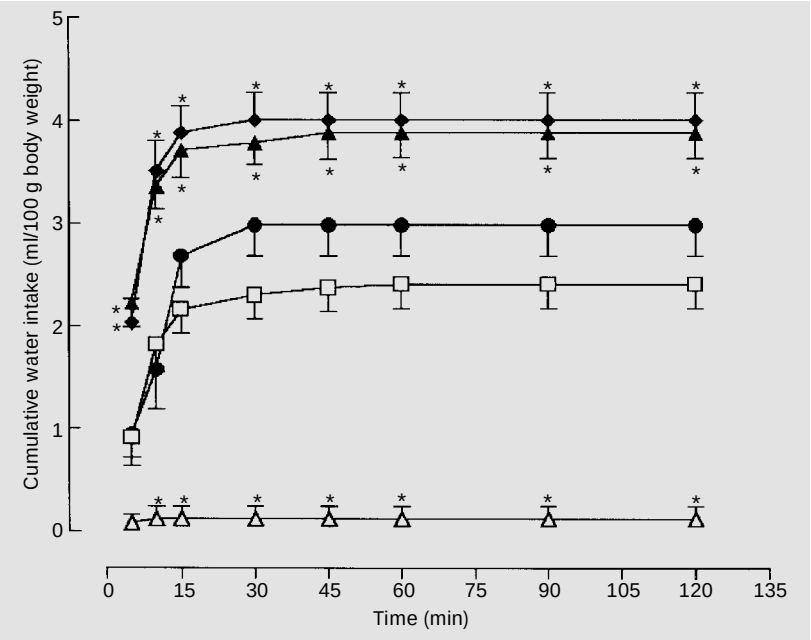

Figure 2. Cumulative water intake (ml/100 g body weight) in hypovolemic rats receiving third ventricle injections of saline (controls) or GR 113808 at different doses. The following groups are represented: hypovol + saline (squares; N = 12); hypovol + GR 20 nmol/rat (filled circles; N = 9); hypovol + GR 40 nmol/rat (filled triangles; N = 9); hypovol + GR 80 nmol/rat (filled lozenges; N = 8). An additional group receiving subcutaneous injections of isotonic saline solution under the same conditions as used for PEG injection plus third ventricle injections of isotonic saline (normovol + saline, open triangles; N = 10) is also shown. Data are reported as means ± SEM. *P<0.05 compared to hypovol + saline (two-way analysis of variance followed by the Student-Newman-Keuls test).

tagonist (10), reduces water intake in animals submitted to a previous salt load and potentiates drinking behavior in animals rendered hypovolemic by subcutaneous PEG administration. This means that central 5-HT_4 receptors may exert a positive drive on water intake due to hyperosmolarity and a negative effect on drinking induced by hypovolemia. Central serotonin participation in water intake is a rather unexplored area. Both stimulatory and inhibitory actions have been reported (6-8). We have previously demonstrated that central 5-HT_1 receptors located in the vicinity of the third ventricle seem to exert a negative effect on water intake in both physiological and pharmacological conditions (11).

Different brain neuronal circuitries control thirst during hyperosmolarity and hypovolemia. Cholinergic pathways seem to trigger water intake after hyperosmolarity while angiotensinergic pathways activate drinking after hypovolemia (12). As the blockade of central 5-HT_4 receptors reduces water intake due to hyperosmolarity, it seems reasonable to suggest that 5-HT_4 activation somehow potentiates cholinergic circuitries related to thirst-generating mechanisms. There are anatomical and neurochemical data to support such interaction, since anatomical connections between serotonergic and cholinergic pathways have been observed (13), central 5-HT_4 receptors increase cholinergic activity (14) and the role of 5-HT_4 receptors in memory processes seems to be mediated by some cholinergic step (15).

On the other hand, the blockade of central 5-HT_4 receptors by GR 113808 potentiated water intake in hypovolemic animals, suggesting that these receptors play an inhibitory role in thirst normally induced by central physiological angiotensinergic activation. As far as we know, evidence of direct connections between central 5-HT_4 receptors and angiotensinergic circuitries is not currently present in the literature. However, it is logical to suggest that 5-HT_4 receptors reduce central angiotensinergic activation due to hypovolemia or impair mechanisms activated by central angiotensin II that lead to the motivational and motor events required for the expression of drinking behavior.

A significant reduction of water intake was observed in hypovolemic animals receiving 40 nmol of GR 113808, while this same dose was devoid of effects when administered to animals with plasma hyperosmolarity. Thus, it seems that the circuitries bearing 5-HT_4 receptors exerting tonic inhibition of water intake during hypovolemia are more easily antagonized than 5-HT_4 receptor-dependent pathways tonically stimulating drinking behavior during hyperosmolarity. This may indicate a greater density of 5-HT_4 receptors linked to stimulation of water intake dependent on cholinergic activation, as compared to the density of receptors involved in the inhibition of drinking behavior mainly induced by an angiotensinergic drive.

We have recently explored the role of central 5-HT_4 receptors in the control of water intake. Using the same selective antagonist employed here (GR 113808) we have demonstrated that 5-HT_4 receptors seem to potentiate water intake due to pharmacological activation of central angiotensinergic pathways, and inhibit drinking produced by central cholinergic activation (6) - a picture that seems to contrast with the data reported here. However, in the present protocol, central cholinergic and angiotensinergic activation was induced by physiological conditions (hyperosmolarity and hypovolemia), while in the previous experimental protocol direct pharmacological stimulation of those circuitries was employed, a substantial difference that may account for the discrepancies observed between the two studies. Indeed, in the present model, hypovolemia and hyperosmolarity sent inputs to the brain, modifying the pattern of activities of other systems related to fluid homeostasis like vasopressin and atrial natriuretic peptide.

Thus, the results obtained here and in our previous study (6) should be interpreted taking into consideration the existence of important differences in the experimental models employed.

In many species including rats, native 5-HT$_4$ receptors are those exhibiting the highest capacity of spontaneous conversion from inactive to active forms, leading to agonist-free induction of intracellular cAMP production and the consequent activation of the cellular machinery, as compared to any other G protein-coupled receptors (16). The processes commanding such endogenous agonist-free self-activation of some receptors are not presently understood. It is possible that the normal physiological conditions promoted here to induce water intake, like hyperosmolarity and hypovolemia, may change the proportions between the active and inactive forms of central 5-HT$_4$ receptors, leading to a different pattern of response after a classical antagonist is used.

Several hypothalamic nuclei containing 5-HT$_4$ receptor mRNA were recently demonstrated (17). Thus, the effects observed here may represent possible actions of serotonin interneurons surrounding the third ventricle linked to important microregulatory effects leading to a precise control of neurovegetative and behavioral parameters. Selective 5-HT$_4$ receptor agonists may provide a novel approach to the treatment of cognitive deficits and selective 5-HT$_4$ antagonists may be useful as anxiolytics or in the treatment of dopamine-related disorders (18). Also, 5-HT$_4$ receptor antagonists are being considered useful in the treatment of urinary incontinence (19). Thus, studies concerning central 5-HT$_4$ functions are opportune and relevant. The data presented here suggest that central 5-HT$_4$ receptors may exert a positive drive on water intake due to hyperosmolarity and a negative input on drinking evoked by hypovolemia.

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References

1. Hoyer D & Martin G (1997). 5-HT Receptor classification and nomenclature: towards a harmonization with the human genome. *Neuropharmacology*, 36: 419-428.
2. Uphouse L (1997). Multiple serotonin receptors: too many, not enough, or just the right number? *Neuroscience and Biobehavioral Reviews*, 21: 679-698.
3. Grossman CJ, Kilpatrick GJ & Bunce KT (1993). Development of a radioligand binding assay for 5-HT$_4$ receptors in guinea-pig and rat brain. *British Journal of Pharmacology*, 109: 618-624.
4. Waeber C, Sebben M, Grossman C, Javoy-Agid F, Bockaert J & Dumuis A (1993). [^{3}H]-GR 113808 labels 5-HT$_4$ receptors in human and guinea-pig brain. *NeuroReport*, 4: 1239-1242.
5. Eglen RM, Wong EHF, Dumuis A & Bockaert J (1995). Central 5-HT$_4$ receptors. *Trends in Pharmacological Sciences*, 138: 391-399.
6. Castro L, De Castro-e-Silva E, Luz CP, Lima AKS, Souza F, Maldonado I, Macêdo DF, Ferreira MG, Santamaria GF, Bandeira IPV, Amor ALM, Carvalho FLQ, Rocha Jr MA & Fregoneze JB (2000). Central 5-HT$_4$ receptors and drinking behavior. *Pharmacology, Biochemistry and Behavior*, 66: 443-448.
7. Neil JC & Cooper SJ (1989). Effects of 5-hydroxytryptamine and *d*-fenfluramine on sham feeding and sham drinking in the gastric fistulated rat. *Physiology and Behavior*, 46: 949-953.
8. Shisheva AC, Ikonomov OC, Stoynev AG & Popova J (1987). Renin release and water-salt balance after central serotonin depletion by p-chlorophenylalanine in Brattleboro and Wistar rats: possible role of ADH. *Endocrinologia Experimentalis*, 21: 219-228.
9. Rowland NE, Li B-H, Fregly MJ & Smith GC (1994). Involvement of angiotensin in water intake induced by peripheral administration of a serotonin agonist, 5-carboxyamidotryptamine. *Brain Research*, 664: 148-154.
10. Gale JD, Grossman CJ, Whitehead JWF, Oxford AW, Bunce KT & Humphrey PPA (1994). GR113808: a novel, selective antagonist with high affinity at the 5-HT$_4$ receptor. *British Journal of Pharmacology*, 111: 332-338.
11. De Castro-e-Silva E, Sarmento C, Nascimento TA, Luz CP, Soares T, Marinho CA, Cunha M, Bulcão C, De Oliveira IR & Fregoneze JB (1997). Effect of third ventricle administration of L-694,247, a selective 5-HT$_{1D}$ receptor agonist, on water intake in rats. *Pharmacology, Biochemistry and Behavior*, 57: 749-754.
12. Johnson AK & Thunhorst RL (1997). The neuroendocrinology of thirst and salt appetite: visceral sensory signals and mechanisms of central integration. *Frontiers in Neuroendocrinology*, 18: 292-353.
13. Khateb A, Fort P, Alonso A, Jones BE &

Mühlethaler M (1993). Pharmacological and immunohistochemical evidence for serotonergic modulation of cholinergic nucleus basalis neurons. *European Journal of Neuroscience*, 5: 541-547.

14. Siniscalchi A, Badini I, Beani L & Bianchi C (1999). 5-HT_4 receptor modulation of acetylcholine outflow in guinea pig brain slices. *NeuroReport*, 10: 547-551.
15. Galeotti N, Ghelardini C & Bartolini A (1998). Role of 5-HT_4 receptors in the mouse passive avoidance test. *Journal of Pharmacology and Experimental Therapeutics*, 286: 1115-1121.
16. Claeysen S, Sebben M, Becamel C, Bockaert J & Dumuis A (1999). Novel brain-specific 5-HT_4 receptor splice variants show marked constitutive activity: role of the C-terminal intracellular domain. *Molecular Pharmacology*, 55: 910-920.
17. Vilarò MT, Cortés R, Gerald C, Branchek TA, Palacios JM & Mengod G (1996). Localization of 5-HT_4 receptor mRNA in rat brain by *in situ* hybridization histochemistry. *Molecular Brain Research*, 43: 356-360.
18. Gaster LM & King FD (1997). Serotonin 5-HT_3 and 5-HT_4 receptor antagonists. *Medicinal Research Reviews*, 17: 163-214.
19. Kumar BB (1984). Urinary incontinence associated with metoclopramide. *Journal of the American Medical Association*, 251: 1553.