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Activation of the serotonergic 5-HT_{1A} receptor in the paraventricular nucleus of the hypothalamus inhibits water intake and increases urinary excretion in water-deprived rats

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ABSTRACT

The paraventricular nucleus (PVN) may be considered as a dynamic mosaic of chemically-specified subgroups of neurons. 5-HT_{1A} is one of the prime receptors identified and there is expressed throughout all magnocellular regions of the PVN. Several reports have demonstrated that a subpopulation of the magnocellular neurons expressing 5-HT_{1A} receptors are oxytocin (OT) neurons and activation of 5-HT_{1A} receptors in the PVN increases the plasma OT. Increasing evidence shows that OT inhibits water intake and increases urinary excretion in rats. The aim of this study was to investigate the role of serotonergic 5-HT_{1A} receptors in the lateral-medial posterior magnocellular region of the PVN in the water intake and diuresis induced by 24 h of water deprivation. Cannulae were implanted in the PVN of rats. 5-HT injections in the PVN reduced water intake and increased urinary excretion. 8-OH-DPAT (a 5-HT_{1A} agonist) injections blocked the water intake and increased urinary output in all the periods of the observation. pMPPF (a 5-HT_{1A} antagonist) injected bilaterally before the 8-OH-DPAT blocked its inhibitory effect on water intake and its diuretic effect. We suggest that antidipsogenic and diuretic responses seem to be mediated *via* 5-HT_{1A} receptors of the lateral-medial posterior magnocellular region of the PVN in water-deprived rats.

1. Introduction

5-HT_{1A} is the most extensively studied serotonin (5-HT) receptor for a number of reasons [1], among the main ones being the availability of a selective agonist 8-OH-DPAT (8-hydroxy-2-(di-*N*-propylamino)tetralin) that allows extensive biochemical, physiological, and pharmacological manipulations [2,3].

The 5-HT_{1A} receptor was the first one among all the 5-HT receptors to be cloned and sequenced [4–6]. Cloning of the 5-HT_{1A} receptor gene revealed that it belongs to the superfamily of G-protein-coupled receptors, harboring 50% amino acid homology with the β_2-adrenergic receptor in the transmembrane domain [4], and the receptor has since then been stably expressed in a number of neural and non-neural cell lines [7,8].

Significant progress has been made in understanding of the function of central nervous system (CNS) 5-HT system in regulation of various pharmacological and physiological functions and behaviors [9,10]. Thus, a tritium ligand, [8H] pMPPF, for 5-HT_{1A} receptors was evaluated by binding studies with rat hippocampal membrane homogenates and autoradiography of rat brain sections. This potential antagonist exhibited high affinity and good selectivity toward 5-HT_{1A} receptors thus providing a useful tool for studies of the 5-HT_{1A} receptor system pharmacology [11,12].

5-HT is present in prosencephalic and rhomboencephalic structures related to the control of drinking behavior and blood pressure and central 5-HT seems to inhibit water intake in several situations. Excitatory and inhibitory systems converge and interact in many ways within body fluid-related neural networks involving different brain neurotransmitters. These include cholinergic, angiotensinergic, and β-adrenergic pathways that induce water intake, and α-adrenergic, opioidergic, and serotonergic pathways that mediate water inhibition [13]. Neuroanatomical tract-tracing techniques and functional mapping methods have implicated numerous brain structures in the processing of systemically derived information related to intra and extracellular volume. Among these structures, the paraventricular nucleus of the hypothalamus (PVN) is an important region in the regulation of fluid electrolytic balance, and various neurotransmitters

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in the PVN are involved in water intake behavior [14–18]. Several studies indicate that serotonergic neurons in the midbrain, but not in more caudal parts of the brain stem, innervate the PVN, and that while the overall density of serotonergic fibers in the PVN is relatively low, their distribution is nevertheless sufficiently distinctive to suggest what their functional significance may be [19]. Experimental data indicate that 8-OH-DPAT-induced release of oxytocin (OT) and adrenocorticotropic hormone (ACTH) are mediated by 5-HT_{1A} receptor – G_z- protein signaling in the PVN [20,21].

Autoradiographic studies indicate a substantial density of [8H] 8-OH-DPAT-labeled 5-HT_{1A} receptors in the ventrolateral magnocellular divisions (containing OT neurons) of the PVN [22]. 8-OH-DPAT is a selective 5-HT_{1A} receptor agonist with high affinity for the 5-HT_{1A} receptor and 10 to 100-fold lower affinity for other 5-HT receptors [23]. It was recently observed that 5-HT_{1A} and 5-HT_{2A} receptors were coexpressed throughout all magnocellular regions of the PVN. A subpopulation of the magnocellular neurons coexpressing 5-HT_{1A} and 5-HT_{2A} receptors are OT neurons [24].

In view of the importance of the PVN serotonergic pathway, we used a pharmacological approach to investigate the role of PVN 5-HT_{1A} receptors on water intake and urinary excretion in water-deprived rats.

2. Experimental procedure

2.1. Animals

Male Holtzman rats weighing 280–320 g were kept in individual stainless steel cages in a room with regulated temperature (23 ± 2 °C) and relative humidity (60 ± 10%), with a 12–12 h light-dark cycle. They were fed on rat pellet chow and water *ad libitum*.

2.2. Brain surgery

Rats were anesthetized with ketamine (80 mg/kg bw) combined with xylazine (7 mg/kg bw) and placed in a stereotaxic instrument. Stainless steel cannulae 0.6 mm (o.d.), 0.33 mm (i.d.) were implanted bilaterally just above the PVN, using the bregma as reference point. Steel cannula introduction points were made in the rats' heads and, at these points, the cranial bone was trepanned with a spherical drill, opening holes with an approximate diameter of 1.0 mm. Stainless steel cannulae were implanted at the following coordinates: 1.8–2.1 mm caudal to the bregma, 0.5–0.7 mm each side of the sagittal line, and 7.6–7.8 mm below the duramater, in conformity with the rat brain atlas of Paxinos and Watson (1986) [25]. The tip of the cannula was positioned at a point 0.5 mm above the PVN. Cannulae were fastened to the skull with acrylic cement and small screws. Insertion of a close-fitting stylet kept the lumen free of debris and clots. Three days before and 3 days after surgery, the animal received prophylactic doses of penicillin. After brain surgery, the animals returned to their individual metabolic cages, with free access to granular chow and tap water for 1 week, until the day of the experiment. After the experiments, the rats were intraperitoneally anesthetized with sodium thiopental (80 mg/kg bw) and dye (2% Evans blue solution, 0.2 μL) was injected into the PVN. Saline followed by 10% buffered formalin was perfused through the heart. The brains were removed, fixed in 10% formalin, frozen, cut in 50-μm sections, stained with Giemsa, and analyzed by light microscopy to confirm the sites of injection into the PVN. The procedures conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. Drugs

Drugs were dissolved in 0.15 M saline solution immediately before each experiment. Injections into rat brains were made using a Hamilton-type syringe (5 μL) connected to a PE-10 polyethylene tube and an injector needle (0.3 mm o.d.) that was 0.5 mm longer than the cannula fixed to the skull. A volume of 0.2 μL was delivered over 20–30 s.

The drugs used were: 5-hydroxytryptamine, (5-HT, Sigma Chemical, St Louis, MO, USA), 8-hydroxy-2-(di-*N*-propylamino) tetralin HBr, (8-OH-DPAT, RBI, Natick, MA, USA, 5-HT_{1A} receptor agonist), 4-(2′-methoxyphenyl)-1-[2′-[*N*-(2″-pyridyl)-*p*-fluorobenzamide] ethyl] piperazine, (pMPPF, RBI, Natick, MA, USA, 5-HT_{1A} receptor antagonist) and d$(CH_2)_5$[Tyr(Me)^2, Thr^4,Orn^5, Tyr $(NH_2)^9$]-vasotocin (Bachem, Saffren Walden, Essex, UK, OT antagonist, ANT-OT).

2.4. Induction and measurement of water intake

Rats had free access to water and food and were put in the metabolic cages at least 5 days before the experiments began. The amount of water ingested in the various experiments was measured with 0.1 mL-graduated glass burettes adapted with a metal drinking spout. Intake was induced by water deprivation during the 24 h that preceded the experiment. Before the experiment, the burettes containing water were removed from the cages.

To observe the effects of 5-HT and 8-OH-DPAT on water intake in water-deprived rats, 10, 20, 40 and 50 μg of 5-HT and 1.25 and 5 μg of 8-OH-DPAT were injected just before offering water burettes to the animals. 5-HT or 8-OH-DPAT doses were used in random order in the experiments. In the first two experiments, the first half were control rats, receiving vehicle injections, and the second half received drug injections with the defined dose. The opposite occurred in the following two experiments: the first half received the drug and the second, saline.

To observe the effects of 5-HT_{1A} antagonist, pMPPF (3.8 μg) and the vehicle (0.15 M NaCl) were administered, either individually or combined, with 20 μg of 5-HT or 2.5 μg 8-OH-DPAT injections. When antagonist was combined, it was injected 10 min before 5-HT or 8-OH-DPAT. Water burettes were offered immediately after brain injections. Intake recordings started immediately after 5-HT, 8-OH-DPAT or vehicle injection and were measured at 30, 60, 90 and 120 min. The interval between successive drug injections was at least 5 days.

2.5. Measurement of urine excretion

Studies were performed to determine whether activation of 5-HT_{1A} receptor mechanisms in the PVN contribute to diuretic responses produced by 8-OH-DPAT and 5-HT in water-deprived rats. Urinary excretion was measured and the same protocols as those used for the water intake were repeated, with the same timing. Urine was collected into polypropylene tubes *via* stainless steel funnels beneath the cages and its volume was measured.

2.6. Statistical analysis

Dependent variables that were not complicated by large differences in variance or normality were analyzed by ANOVA II. Tukey's honestly significant difference test was used for post hoc comparisons. Some intake data were analyzed by nonparametric tests because of the large differences in variance or normality between groups. First, a Kruskal–Wallis ANOVA of ranks was used across the groups at each time interval. When that test was significant, we proceeded to examine all pairwise comparisons with the aid of the Mann–Whitney test. Tests were conducted on the accumulated data at 15, 30, 60, 90 and 120 min. Data are presented as mean standard error (SE). A probability of <0.05 was required for significance.

3. Results

3.1. Drinking induced by 5-HT and 8-OH-DPAT into the PVN

The PVN parameters studied were observed in subjects ($n=148$) that had the correct injector placement points concentrated in the