Role of Various Types of Serotonin Receptors in Regulation of Drinking Behavior and Salt Appetite in Vasopressin-Deficient Brattleboro Rats

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Serotonin 5-HT_{1A} receptor agonist 8-OH DPAT suppressed drinking behavior in Brattleboro and Wistar rats. 5-HT_{1B} agonist CGS-12066A and 5-HT_{2A} antagonist ketanserin did not affect drinking behavior in Brattleboro rats; 5-HT_3 antagonist ondansetron suppressed water consumption and 5-HT_{1A} agonist stimulated salt appetite in Brattleboro, but not in Wistar rats. Presumably, vasopressin regulates thirst and salt appetite by modulating sensitivity/density of various types of 5-HT receptors.

Key Words: *5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_3 receptors; drinking behavior; salt appetite; Brattleboro rats*

Serotonin (5-HT) receptors are involved in the regulation of water and salt consumption [4,5,10,12]; moreover, 5-HT affects production of vasopressin, the main hormone regulating water-salt balance [1,2,6,7]. However, the effect of vasopressin on the cerebral 5-HT system is poorly understood. We previously demonstrated that genetically determined vasopressin deficiency modified the reaction of the hypothalamic 5-HT system to water deprivation and hydration. It was hypothesized that vasopressin modulates 5-HT metabolism in the brain [11]. This assumption agrees with the presence of vasopressin in the dorsal raphe nuclei, the major structure containing bodies of 5-HT neurons [8]. The effect of vasopressin on the reactions of 5-HT receptor system is not clear. There are seven types of 5-HT receptors [3]. 5-HT_1, 5-HT_2, and 5-HT_3 attract special attention, as selective agonists and antagonists were synthesized for these receptors and these three types of 5-HT receptors differ in secondary messenger [9]: adenylate cyclase for 5-HT_1 and inositol phosphate for 5-HT_2, 5-HT_3 functions without second messenger and represents a cationic channel for Na^+, K^+, and Ca^+. At present, 5 subtypes of 5-HT_1 receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F}) and 3 subtypes of 5-HT_2 receptors (5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}) were cloned and sequenced.

We investigated the effects of 5-HT_{1A} receptor agonist and antagonist, 5-HT_{1B} receptor agonist, 5-HT_{2A} and 5-HT_3 receptor antagonists on drinking behavior and salt appetite in Brattleboro rats. These rats are characterized by the absence of vasopressin synthesis due to single nucleotide deletion in the gene encoding this hormone [13].

MATERIALS AND METHODS

Adult male Brattleboro rats (250 g) were kept under standard vivarium conditions. Two days before the experiment the animals were deprived of water (but received standard fodder and dry bread). Experimental rats were intraperitoneally injected with test drugs and controls received normal saline or solvent and after 20 min water and 1.8% NaCl solution were offered for free choice. The volumes of consumed water and saline were measured every 30 min for 1 h. Effective doses of test drugs for Wistar rats were determined in preliminary experiments and these rats were then used as controls.

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5-HT_{1A} receptor agonist 8-OH-DPAT was injected to Brattleboro and Wistar rats in a dose of 1.0 mg/kg, 5-HT_{1A} receptor antagonist pMPPI in a dose of 0.4 mg/kg, 5-HT_{1B} receptor agonist CGS-12066A maleate in a dose of 1.0 mg/kg (all drugs from Research Biochemicals International), 5-HT_{2A} receptor antagonist ketanserin (Janssen Pharmaceutica) in a dose of 3.0 mg/kg, and 5-HT_3 receptor antagonist ondansetron (Glaxo) in a dose of 0.5 mg/kg. Ketanserin and ondansetron were dissolved in distilled water, other agents were dissolved in normal saline.

The results were processed by ANOVA using Microcal Origin 5.0 and Statistica for Windows 5.0 software.

RESULTS

Selective 5-HT_{1A} agonist 8-OH-DPAT in a dose suppressing drinking behavior in Wistar rats (1 mg/kg) significantly decreased water consumption in Brattleboro rats. This effect persisted for 1 h. At the same time, the drug markedly increased salt appetite in Brattleboro, but not in Wistar rats. The effect peaked during the second half-hour: salt consumption increased almost 3-fold (to 12.20±1.11 *vs.* 4.40±0.83 in the control, $p<0.001$) and water consumption decreased 5-fold (to 1.40±0.29 *vs*. 7.10±1.36 in the control, $p<0.001$), whereas in Wistar rats the inhibitory effect manifested within the first 30 min. Blockade of 5-HT_{1A} receptors with pMPPI increased water consumption in Wistar rats, but did not change drinking behavior or salt appetite in Brattleboro rats (Table 1). 5-HT_{1B} agonist CGS-12066A in a dose increasing water consumption in Wistar rats (1 mg/kg) did not change drinking behavior or salt appetite (both $p>0.05$) in Brattleboro rats. Selective 5-HT_{2A} receptor antagonist ketanserin (suppressing drinking behavior of Wistar rats) also had no effect on salt and water consumption in Brattleboro rats. 5-HT_3 receptor blocker ondansetron in a dose of 0.5 mg/kg did not modify salt appetite, but decreased water consumption during the first 30 min ($p<0.05$).

Hence, mutation responsible for vasopressin deficiency in Brattleboro rats radically changes the reaction of all types of receptors to water deprivation. In Wistar rats different subtypes of 5-HT_1 and 5-HT_2 regulators and 5-HT_3 receptors are involved in the regulation of thirst mechanisms, and their effects on these mechanisms are different: 5-HT_{1B} and 5-HT_{2A} receptors activate drinking behavior, while subtype 5-HT_{1A} and type 5-HT_3 inhibit it. Activation of 5-HT_{1A} receptors suppressed drinking behavior in vasopressin-deficient Brattleboro rats and Wistar rats, and increased salt appetite in Brattleboro, but not in Wistar rats. It is noteworthy that in Brattleboro rats the effect developed more slowly than in Wistars. Blockade of 5-HT_{1A} receptors had no effect on water and salt consumption. Activation of 5-HT_{1B} and blockade of 5-HT_{2A} receptors did not essentially affect the volume of consumed water or saline. Blockade of 5-HT_3 receptors markedly suppressed drinking behavior in Brattleboro rats and stimulated it in Wistar rats.

Hence, only two of the studied 5-HT receptor types modify drinking motivation in vasopressin-deficient animals. Similarly to Wistar rats, the 5-HT_{1A} subtype is inhibitory and unlike Wistar rats, the 5-HT_3 type is presumably activating. Moreover, 5-HT_{1A} receptors are involved in the regulation (activation) of salt appetite of Brattleboro rats. The findings on different contribution of 5-HT receptors into the regulation of drin-

TABLE 1. Effects of Selective 5-HT Receptor Agonists and Antagonists on Water and Saline Consumption (ml) by Wistar and Brattleboro Rats during the First 30 min of Experiment (*M*±*m*)

Experimental series	Wistar		Brattleboro	
	water	NaCl	water	NaCl
8-OH-DPAT, 1.0 mg/kg	6.50±0.97****	1.5±0.4	3.30±0.53*	7.90±1.64
	11.60±2.15	2.30±0.53	13.00±1.36	5.70±1.03
pMPPI, 0.4 mg/kg	12.2±1.2****	3.40±1.12	14.70±0.65	4.90±1.03
	9.30±0.64	3.80±0.44	13.00±1.36	5.70±1.03
CGS-12066A, 1.0 mg/kg	13.10±1.22**	4.70±0.62	7.70±2.17	5.70±1.32
	9.30±0.64	3.80±0.44	9.6±1.9	6.20±1.06
Ketanserin, 3.0 mg/kg	6.0±0.8**	5.20±0.55	11.60±0.88	5.20±0.92
	10.10±0.81	5.4±0.6	13.30±1.03	5.80±0.79
Ondansetron, 0.5 mg/kg	15.10±0.54*	4.70±0.97	12.50±1.21****	5.60±0.94
	10.10±0.81	5.4±0.6	15.80±0.65	5.30±0.68

Note. Numerator: experiment; denominator: control. *$p<0.001$, **$p<0.01$, ***$p<0.01$, ****$p<0.05$ compared to the corresponding control.

king behavior and salt appetite in Wistar and Brattleboro rats indicate that vasopressin modulates not only 5-HT metabolism [11], but also sensitivity or, probably, density of 5-HT receptors in brain structures, thus playing an important role in the regulation of salt appetite and drinking behavior.

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