Comparative Analysis of Dipsogenic Effects of Systemic and Intracerebral Injection of Angiotensin II to Rats after Carotid Glomectomy

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Systemic administration of angiotensin II after carotid glomectomy produced a less pronounced dipsogenic effects (consumption of water and NaCl solution) compared to sham-operated control animals. Injection of angiotensin II into the lateral cerebral ventricles of the same glomectomized rats increased water and NaCl consumption to a level surpassing that of sham-operated animals. The number of drinking acts and comfortable grooming acts decreased in glomectomized animals after systemic administration of angiotensin II, but increased after its intracerebral injection compared to the control. The results confirm the hypothesis that carotid chemoreceptors, as the peripheral component of the renin-angiotensin system, participate in the mechanisms of angiotensin-induced thirst, "salt appetite", and associated behavioral forms (comfortable grooming) synergically with the central cerebral receptors.

Key Words: *water-salt balance; renin-angiotensin system; angiotensin II; carotid body chemoreceptors; carotid glomectomy*

Autoregulation of water-salt homeostasis is maintained by systemic interactions of the central and peripheral components of the renin-angiotensin system (RAS). Angiotensin II (A-II), the main effector peptide of RAS participating in thirst and "salt appetite" initiation, is involved in the mechanisms maintaining water-salt balance and osmotic blood pressure [3,11,13,15].

The involvement of A-II-sensitive receptors of the peripheral vascular system in the thirst mechanisms is little studied. One of the major peripheral vascular chemoreceptors is the carotid body, whose receptors reacting to changes of gas constants, osmotic parameters of the blood, and other components of the water-salt balance send afferent signals about chemical composition of the blood into cerebral structures [1,4,8,10,12]. Discovery of A-II-sensitive receptors in the carotid body sensory cells [9,14] implies their involvement into the RAS-controlled regulation of water and mineral salt levels in the body [7]. We found that bilateral surgical removal of the carotid bodies (carotid glomectomy; CGLE) in rats was associated with an increase in the mean daily consumption of NaCl solution. Thirst motivation and "salt appetite", usually induced by intraperitoneal injection of A-II, did not manifest or were significantly reduced in rats after CGLE [7,11, 13]. The study of the characteristics of A-II-induced thirst after injection of A-II into the brain of glomectomized rats seems essential for understanding of the causes of suppressive effect of CGLE on the dipsogenic effect of A-II after systemic administration.

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We compared the objective characteristics of thirst and salt appetite in glomectomized rats after intraperitoneal and intracerebroventricular injection of A-II.

MATERIALS AND METHODS

Experiments were carried out on 14 male Wistar rats initially weighing 292.9±13.0 g. During the entire experiment the rats in individual boxes were allowed free access to two burettes containing water and 1% NaCl solution, respectively, and to standard granulated fodder. Daily volumes of drunk fluid were measured in glomectomized (experimental) and sham-operated (control) animals over 2 weeks before and 2 weeks after the intervention. The methods were described in detail [2,7]. After 4 weeks the control and experimental animals were intraperitoneally injected with A-II (Sigma; 300 μg/kg in 1 ml saline) and volumes of water and NaCl solution drunk over 60 min (min 0-10, 10-20, 20-40, and 40-60) after the injection were measured. The numbers of drinking acts and grooming acts (comfort "hygienic" behavior) during this period were counted. After 1 week the rats intraperitoneally injected with A-II were scalped under ether narcosis and cannulas were implanted into the lateral cerebral ventricles and fixed to the skull with protacryl. Two days after implantation of the cannulas A-II was injected (300 ng in 3 μl, unilaterally) into the cerebral lateral ventricle with a Hamilton syringe. The volume of fluid drunk after intracerebroventricular injection was measured during the same period as after intraperitoneal injection. The numbers of drinking and grooming acts were recorded similarly as after systemic administration of A-II.

The volume of consumed fluid was expressed in ml/100 g. The significance of differences was evaluated using Mann—Whitney *U* test.

RESULTS

Two weeks after CGLE we observed changes in the volume of consumed fluid. The mean daily volume (for 2 weeks) of NaCl solution drunk increased after the intervention in 5 of 7 rats; in 2 rats the mean volume of water drunk during a day surpassed that before surgery. The increase in the consumption of NaCl solution was in line with our previous data obtained under similar experimental conditions [7].

Glomectomy suppressed the typical dipsogenic effect of intraperitoneal A-II similarly to previous experiments [7]. Analysis of the dynamics of water and NaCl consumption over 1 h after intraperitoneal injection of A-II showed that these parameters were lower in glomectomized rats compared to sham-operated controls throughout the observation period (Fig. 1). The number of drinking acts and the number of grooming acts after injection of A-II in experimental rats were significantly lower than in controls (Fig. 2, *a*).

Intracerebroventricular injection of A-II to glomectomized rats (used in previous experiments with systemic administration of the peptide) induced thirst and salt appetite (Fig. 3, *b*). Experimental rats drank significantly more water than control animals during the first 10 min and 10-20 min after injection (Fig. 3, *a*, *b*; $p<0.01$). The volume of NaCl solution drunk over the period of 40-60 min after injection was greater in glomectomized rats compared to sham-operated ones (Fig. 3, *a*, *b*). In experimental

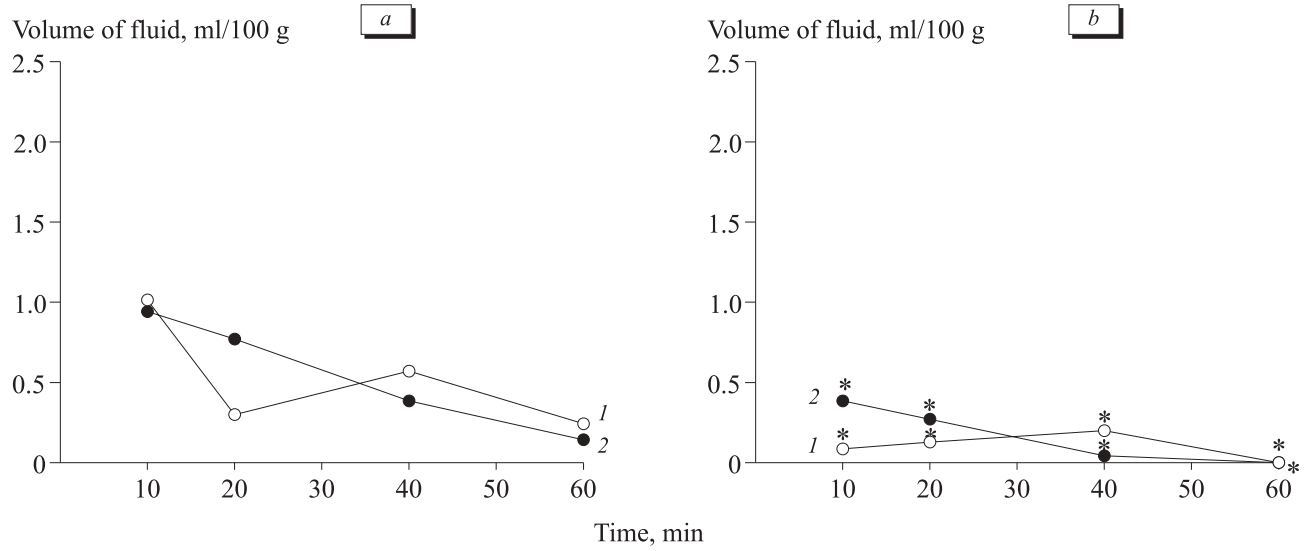

Fig. 1. Suppression of dipsogenic effect of intraperitoneal injection of A-II in rats after carotid glomectomy. Here and in Fig. 3: *a*) control (sham operation); *b*) experiment (carotid glomectomy). Here and in Figs. 2, 3: **p*<0.01 compared to the control.

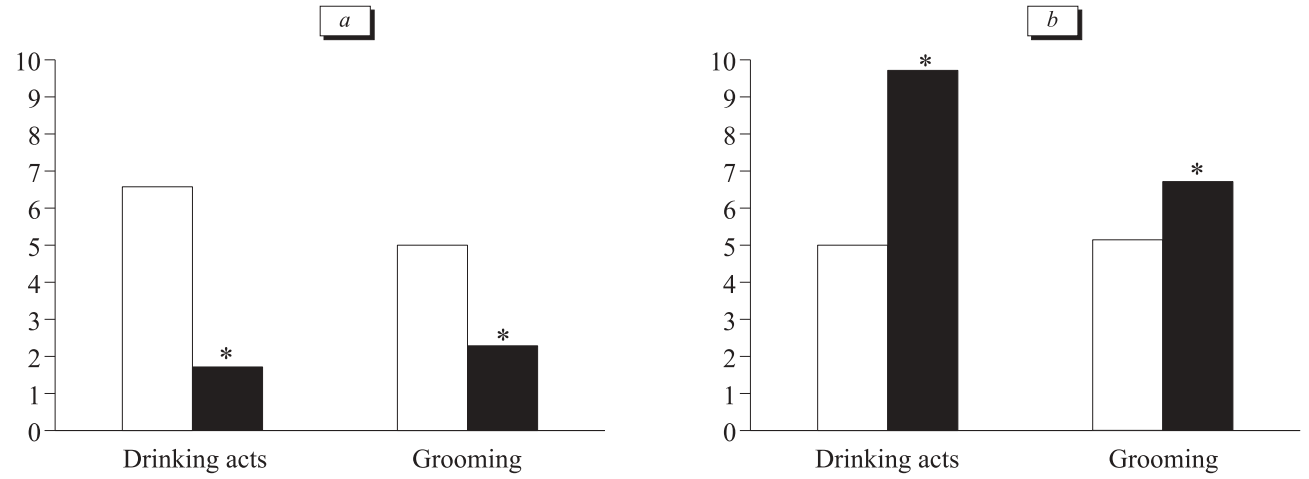

Fig. 2. Number of drinking acts and grooming acts after intraperitoneal (*a*) and intracerebroventricular (*b*) injections of A-II to glomectomized rats. Light bars: control (sham operation); dark bars: experiment (carotid glomectomy).

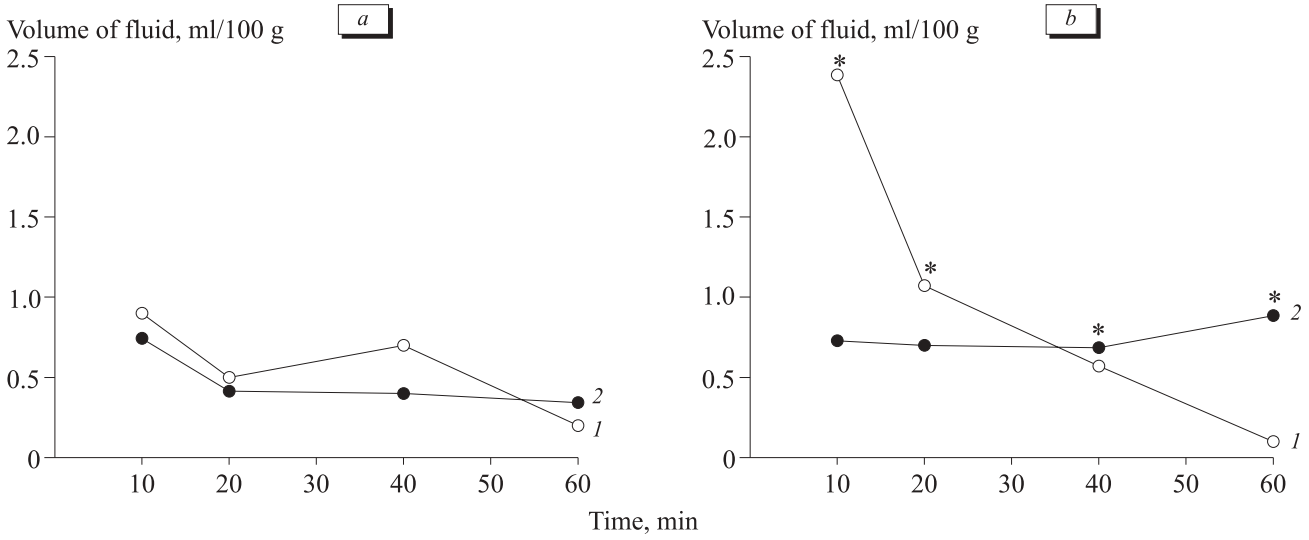

Fig. 3. Increase of dipsogenic effect of intracerebroventricular injection of A-II after carotid glomectomy.

animals the summary values of water and NaCl solution consumption over 1 h after injection of A-II were higher than in controls. The numbers of drinking and grooming acts in glomectomized rats were higher than in sham-operated animals (Fig. 2, *b*; $p<0.01$).

Our previous studies of the effect of CGLE on A-II-induced thirst [7], and the data on the presence of A-II-sensitive receptors in the carotid glomus cells and potentiating effect of this peptide on afferent activity of the sinocarotid nerve [9,14] confirmed the hypothesis on the important role of carotid body chemoreceptors in the mechanisms of water-salt balance regulation with participation of RAS. Our previous experiments showed significant suppression of thirst and salt appetite induced by systemic injections of A-II in glomectomized rats [7]. In order to detect the mechanisms of suppression of A-II-induced thirst and salt appetite after systemic injection of the peptide to glomectomized animals we compared the dipsogenic effects after intraperitoneal and intracerebroventricular injections of A-II to glomectomized rats. When analyzing the decrease in fluid consumption, number of drinking acts and grooming acts after systemic treatment with A-II we can conclude with certainty that in animals subjected to CGLE no thirst was induced by A-II or this thirst was strongly suppressed. Injection of the peptide into the cerebral ventricles of the same rats caused thirst motivation and salt appetite surpassing the control level. The significant increase in the volume of water consumption within the first 10 min postinjection and increased the number of drinking acts and hierarchically related to them grooming acts in glomectomized rats during the first hour in comparison

with sham-operated animals attest to rapid formation and realization of intensive thirst motivation after intracerebral injection of A-II to glomectomized rats.

The changes observed in the behavior of glomectomized animals can be mediated by two mechanisms. Activation of chemoreceptor cells of the carotid glomus with A-II enhances afferentation from the sinocarotid nerve, which leads to further activation of neurons of the medulla oblongata solitary tract nuclei and the network of cerebral RAS neurons [9,11,13,15]. In parallel, A-II circulating in the blood penetrates into brain structures and synergically stimulates RAS neurons. Both mechanisms are united in the central-peripheral integration of thirst and salt appetite motivation. Presumably, the former mechanism predominates after systemic injection of A-II, because A-II-induced thirst does not manifest in glomectomized rats. After central injections A-II seems to diffuse in the cerebrospinal fluid, easily binds to angiotensin receptors of RAS neurons [11,13,15], whose activation leads to dipsogenic effect without participation of the former mechanism. The increase in the dipsogenic effect in glomectomized rats in comparison with control rats can be due to sensitization of brain neurons to A-II as a result of the absence of afferent signaling from the carotid glomus chemoreceptors.

Sham-operated controls drank the same volumes of water and NaCl solution after systemic and intracerebroventricular injections of A-II during the entire period of observation (Fig. 1, *a*; 3, *a*). Glomectomized rats drank more water during the first 10 min and more NaCl solution during min 40-60 after injection of A-II into the brain ventricle (Fig. 3, *b*; $p<0.01$). We consider that these facts indicate the involvement of the peripheral gustatory receptor system determining water or NaCl solution preference in the realization of A-II-induced thirst as a regulator of the volume of osmotic components of body fluids entering from the outside [5,6].

Hence, comparative analysis of the dipsogenic effects of systemic and intracerebroventricular injections of A-II to glomectomized rats showed that carotid body receptors (peripheral components of RAS) activated by A-II circulating in the blood, initiate the need in water and NaCl solution synergically with angiotensin activation of RAS brain structures. These total systems central-peripheral chemoreceptor interactions maintain the water-salt balance under RAS control.

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