Free and Protein-Bound Angiotensin II_{1-7} in the Regulation of Drinking Behavior and Hemodynamics in Rats

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We compared physiological activity of synthetic analogues of endogenous protein-peptide compounds, complexes of angiotensin II_{1-7} with functionally different proteins (transport protein, serum albumin; and neurospecific Ca^{2+}-binding protein, S100b). Physiological activity of angiotensin II_{1-7} was shown to depend on the type of a carrier protein. Our results suggest that complexes of angiotensins with BSA and S100b are strong factors for the integration of central and peripheral functions at the homeostatic and behavioral level.

Key Words: *angiotensin II_{1-7}; neurospecific protein S100b; serum albumin; hemodynamics; drinking behavior*

Renin-angiotensin system (RAS), one of the major regulatory systems in the body, plays an important role in adaptive and compensatory processes (*i.e.*, maintenance of water-salt balance and cardiovascular functions; modulation of humoral and cellular immunity) [7,12,14,15]. The main effector peptide of RAS, angiotensin II (AT-II), is formed during enzymatic processing of angiotensinogen precursor peptide and metabolized by aminopeptidases A and N, carboxypeptidase P, and angiotensin-converting enzyme (ACE). This process is accompanied by the formation of peptide end-products, including AT-II_{1-7} fragment, AT-III, and AT-IV. Little is known about physiological activity of these peptides [6,7,12,14]. Much attention is paid to AT-II_{1-7}, which serves as a functional antagonist of AT-II. AT-II_{1-7} is a product of alternative splicing; it can be formed from AT-I and/or AT-II with the involvement of an ACE isoform (ACE-2), and has specific receptors (*Mas* receptors). At the same time, AT-II_{1-7} exhibits affinity for AT_1 and AT_2 receptors for AT-II [9,11,13].

We previously compared the effects of free AT-II and AT-IV and protein-peptide complexes (PPC) of these substances and described for the first time physiological activity of these complexes. This activity was strongly modulated by functionally different proteins in the composition of PPC [2,3]. We hypothesized that these "chimeric" compounds play a role in not only prolonged maintenance of certain functional states, but also the reproduction of relevant inherited and acquired behavioral skills [1,4].

Due to functional antagonism of AT-II_{1-7} and AT-II, this fragment holds much promise for the therapy of pathologies accompanied by RAS hyperactivation (*e.g.*, hypertension, atherosclerosis, nephropathy, and diabetes) [6,7,13].

Here we compared the role of free AT-II_{1-7} and AT-II_{1-7} bound to functionally different proteins (transport protein, serum albumin; and neurospecific Ca^{2+}-binding protein, S100b) in the formation and realization of complex acquired drinking behavior in rats and evaluated the effect of AT-II_{1-7} complexes on hemodynamic parameters (SBP and HR).

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 300-400 g.

Synthetic conjugates of AT-II_{1-7} (American peptides) with BSA (Sigma) and S100b protein (Sigma)

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were synthesized in our laboratory. Synthetic compounds serve as model analogues of endogenous PPC. PPC of AT-II_{1-7} and carrier protein molecules were synthesized by the carbodiimide method. Chromatography showed that these conjugates contain 10-12 and 5-6 molecules of AT-II_{1-7} per one BSA and S100b molecule, respectively.

Physiological activity of AT-II_{1-7}–BSA and AT-II_{1-7}–S100b complexes was compared with that of native AT-II_{1-7}. We studied the effect of intraperitoneal treatment with these complexes on drinking behavior and hemodynamic parameters (SBP and HR).

Experimental animals of the treatment groups received native AT-II_{1-7} (300 μg/kg) and AT-II_{1-7}–BSA and AT-II_{1-7}–S100b complexes in doses equivalent to 300 μg/kg AT-II_{1-7}. Control rats received intraperitoneal injections of the test substances in combination with an ACE inhibitor Capoten (n=9), direct antagonist of AT_1 receptors losartan (n=8), and native AT-II (n=11) in a dose of 300 μg/kg. Activity of test substances was compared with that of physiological saline.

The effect of PPC consisting of AT-II_{1-7} and proteins on drinking behavior of untrained animals that had free access to water was evaluated by the volume of water intake over 3 h after injection. We also analyzed the influence of PPC including AT-II_{1-7} on the formation and performance of a complex operant drinking behavior. The duration of successive stages of drinking operant behavior in rats was evaluated on an automatic device [5]. Hemodynamic parameters of awake rats were studied in plastic cages by the indirect method with a NIBP system (AD Instruments). Variations in SBP and HR were expressed as a percentage of the baseline. The values of SBP and HR were interpolated with cubic splines for adjusting the data to univariate time series.

All experiments were conducted according to the Helsinki declaration on the welfare of animals.

The results were analyzed using Student's t test.

RESULTS

AT-II_{1-7} in PPC with functionally different proteins was shown to have various physiological properties. Carrier proteins specifically modulate their effects on behavior and hemodynamics.

Intraperitoneal injection of AT-II_{1-7} (n=8), AT-II_{1-7}–BSA (n=9), and AT-II_{1-7}–S100b (n=8) was not accompanied by induction of water intake in animals under conditions of free access to water, while in water-deprived rats free and BSA-bound AT-II_{1-7} stimulated water intake, similarly to AT-II. At the same time, free AT-II_{1-7} (n=10), AT-II_{1-7}–BSA (n=8), and AT-II_{1-7}–S100b (n=8) significantly inhibited drinking behavior in rats that were pre-trained in a complex drinking operant skill in an automatic device (p<0.05; Fig. 1, *a*) and had no effect on training a new skill for getting reinforcement in an automatic device (change in direction of manipulative disk rotation to obtain water; Fig. 1, *b*).

Analysis of the peripheral effects of free and protein-bound AT-II_{1-7} showed that administration of free AT-II_{1-7} (n=10) had little effect on SBP and HR (similar to injection of 0.9% NaCl in control animals; Fig. 2, *a*). AT-II_{1-7}–S100b (n=7) produced a strong hypertensive effect (by both the amplitude and duration of action; Fig. 2, *a*). Single injections of AT-II_{1-7}–S100b to rats were followed by a significant increase in SBP (p<0.05 compared to the control treatment with 0.9% NaCl). The observed changes persisted for a long time (1.5 h). By contrast, AT-II_{1-7}–BSA (n=8) had little effect on these parameters.

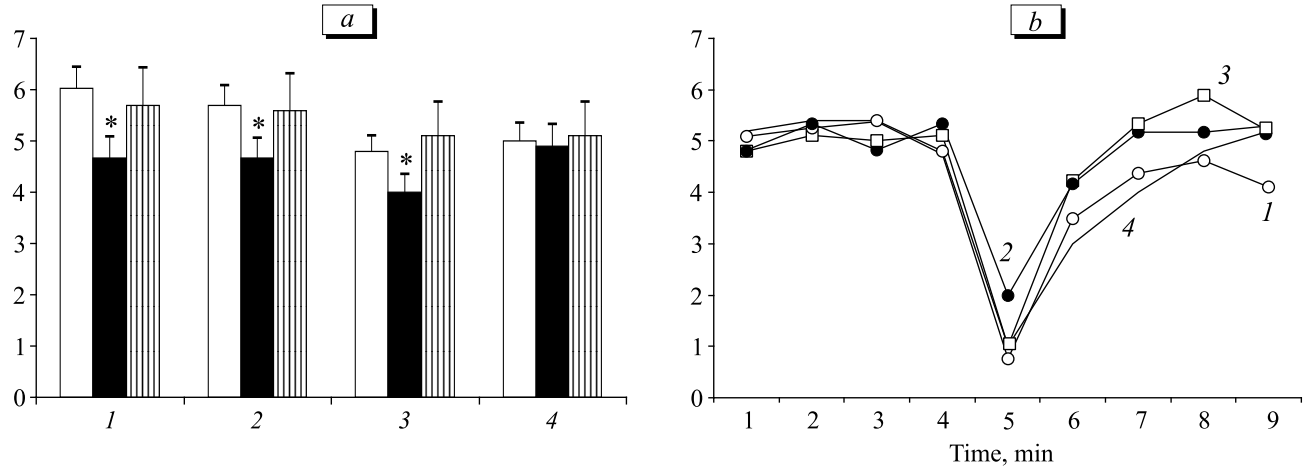

Fig. 1. Effects of free and protein-bound AT-II_{1-7} on the performance (*a*) and development (*b*) of operant drinking behavior in rats. Ordinate: number of resultant episodes. Light bars, baseline; dark bars, administration of substances; shaded bard, aftereffect. Here and in Fig. 2: *a*, *b*: administration of free AT-II_{1-7} (*1*); AT-II_{1-7}–BSA (*2*); AT-II_{1-7}–S100b (*3*); and 0.9% NaCl (*4*). *p<0.05 compared to the baseline.

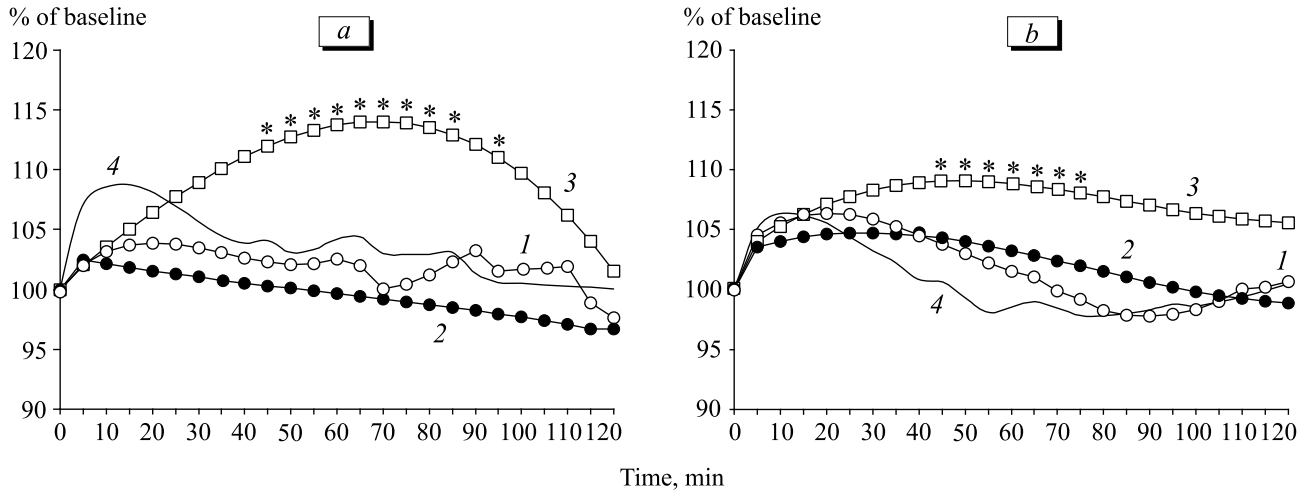

Fig. 2. Changes in SBP (*a*) and HR (*b*) after intraperitoneal injection of free and protein-bound AT-II_{1-7}.

ACE inhibitor Capoten and direct antagonist of AT_1 receptors losartan did not modulate the effect of free AT-II_{1-7}, AT-II_{1-7}–BSA, and AT-II_{1-7}–S100b on hemodynamic parameters.

The dipsogenic and hypertensive effects of AT-II were significantly reduced after combined treatment with AT-II and AT-II_{1-7}.

Changes in HR were minor; however, treatment with AT-II_{1-7}–S100b was followed by insignificant, but prolonged tachycardia (Fig. 2, *b*).

Our results indicate that the fragment of AT-II_{1-7}, which serves as a functional antagonist of AT-II, has intrinsic dipsogenic activity. This activity is specifically observed in water-deprived animals and manifested in an additional stimulation of the inherited drinking behavior. AT-II_{1-7} does not induce water intake in animals that have free access to water.

In contrast to AT-II, free AT-II_{1-7} and AT-II_{1-7} bound to functionally different proteins (transport protein BSA and neurospecific protein S100b) do not activate the acquired drinking behavior. Therefore, this fragment serves as a functional antagonist of AT-II at the behavioral level.

At the level of hemodynamics regulation, binding of AT-II_{1-7} to BSA stabilizes AT-II_{1-7} properties as a functional antagonist of AT-II, while binding of AT-II_{1-7} to S100b contributes to the acquisition of antagonistic properties to AT-II.

It can be hypothesized that physiological activity of angiotensins during the interaction with various types of specific receptors is mediated by heterogeneous processes of intracellular signal transduction [7,8,10,14,15]. These changes contribute to a specific regulatory response, which involves the cell genome. Complexes of angiotensins with functionally different proteins probably provide the divergence of signal transduction pathways, which induces an adequate cell response in the whole body with the involvement of RAS.

Various proteins (transport protein BSA and neurotrophic protein S100b) modulate activity of angiotensins (AT-II_{1-7}, AT-II, and AT-IV), which may contribute to cooperation or antagonism between some components of RAS in central and peripheral integration of functions. One of these components (AT-II_{1-7}) probably plays a reserve role in the maintenance of hemodynamics and regulation of behavior under extreme conditions.

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