Physiological Effects of Complexes of Angiotensins with Functionally Different Carrier Proteins

E. I. Pevtsova, S. M. Tolpygo, M. F. Obukhova, and A. V. Kotov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 8, pp. 135-138, August, 2008
Original article submitted November 16, 2007

We compared activity of synthetic complexes of angiotensin II and functionally different proteins (transport protein, serum albumin and neurospecific Ca^{2+}-binding protein S100b) as analogues of endogenous protein—peptide complexes. Physiological activity of angiotensin II was specifically modified by these proteins. It was hypothesized that the complex of angiotensin II and S100b is primarily involved in the regulation of hemodynamics, whereas the complex of angiotensin II and bovine serum albumin plays a role in the formation and realization of drinking behavior.

Key Words: *angiotensin II; neurospecific protein S100b; serum albumin; hemodynamics; drinking behavior*

The renin-angiotensin system plays a key role in the regulation of hemodynamics, water-salt metabolism, and mechanisms of thirst. Dysfunction of this system accompanies a variety of chronic diseases, including arterial hypertension, atherosclerosis, ischemia of the heart and brain, and mineral metabolism disorders [2,6,10,15].

Our previous studies showed that in contrast to free angiotensin synthetic protein—peptide complexes (PPC) of angiotensins are immunoreactive and more polyfunctional, they produce prolonged and specific effects, particularly on acquired behavior formed on the structural and functional basis of thirst motivation [1].

Functions of endogenous PPC are evaluated in modern molecular, biological, and biochemical studies. Published data suggest that endogenous PPC are involved in the pathogenetic mechanisms of so-called "conformational diseases" [2,10,12,14]. Little is known about the role of free and protein-bound peptide components of the renin—angiotensin system (angiotensins) in modification and possible transformation of physiological processes into pathological processes.

Molecules of serum albumin are characterized by high conformational mobility and reversible binding of a variety of endogenous and exogenous ligands (hormones, peptides, vitamins, *etc*.) [8]. Serum albumin is also the major factor determining oncotic pressure and regulating endothelial permeability and stability of blood—tissue barriers. Serum albumin is a chaperone protein in receptor endocytosis, transcytosis, and inflammation [8], which has no effect on the behavior. By contrast, neurospecific protein S100b has a strong modulatory effect on learning and memory [7,9,13]. This protein regulates the growth, differentiation, and development of brain cells. Moreover, S100b serves as a marker of several pathological processes. Neurodegenerative and hemodynamic disorders are accompanied by significant changes in the concentration of S100b protein [4,11].

Here we compared physiological properties of the major effector peptide of the renin—angiotensin system (angiotensin II, AT-II) in PPC with functionally different carrier proteins, including serum albumin (main transport protein in blood plasma) and Ca^{2+}-binding neurospecific protein (S100b protein).

P. K. Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** lab_motiv@mail.ru. S. M. Tolpygo.

0007-4888/08/1462-0172

MATERIALS AND METHODS

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

Synthetic conjugates of AT-II (MP Biomedicals) with bovine serum albumin (BSA) or S100b protein (Sigma) were synthesized in our laboratory. These compounds served as model analogues of endogenous PPC. PPC of AT-II and carrier protein molecules were synthesized using a bifunctional linking agent 1-cyclohexyl-3(2-morpholine-ethyl)-carbodiimide (Sigma). Chromatography and radioisotope assay with I^{125}-labeled AT-II showed that these conjugates contain 10-12 and 5-6 molecules of AT-II per BSA and S100b molecules, respectively.

Physiological activity of AT-II-BSA and AT-II-S100b complexes was estimated after intraperitoneal injection and compared with that of native AT-II. We studied the effect of complexes on drinking behavior and hemodynamic parameters (systolic blood pressure, SBP; and heart rate, HR).

The animals of the treatment groups received native AT-II (300 μg/kg, n=22), PPC of AT-II and BSA (n=25), and PPC of AT-II and S-100b (n=22). The doses of PPC were equivalent to 300 μg/kg AT-II. Control rats received intraperitoneal injections of the test substances in combination with angiotensin-converting enzyme inhibitor Capoten (n=19), competitive antagonist of angiotensin AT_1 receptors saralasin (n=17), and direct antagonist of AT_1 receptors losartan (n=17) in a dose of 300 μg/kg. Activity of the test substances was compared with that of solvent (0.9% NaCl, n=19).

The effects of PPC of AT-II and proteins on drinking behavior were studied on non-thirsy animals. The volume of water intake was measured over 3 h after injection. Hemodynamic parameters were recorded by the indirect method using a NIPB system (ADInstruments). Awake rats were placed in plastic cages. Because of variability in baseline SBP and HR, changes in these parameters were expressed in percent of initial value.

The results were analyzed by Student's t test.

RESULTS

AT-II in PPC with various carrier proteins exhibited different physiological activity. Moreover, significant differences were revealed in central and peripheral manifestations of AT-II activity.

Intraperitoneal injection of AT-II (n=10) and PPC of AT-II with BSA (n=10) and S100b (n=10) had a central effect of different degree, which was manifested in water consumption by non-thirsty animals. Drinking behavior was most pronounced after injection of free and BSA-bound AT-II. It should be emphasized that the complex of AT-II and BSA was more potent in this respect. The latency of drinking behavior was 9-12 min and its duration after injection of native AT-II and PPC of AT-II with BSA was 20-30 and 50-70 min, respectively. However, the latency of drinking behavior was much longer after injection of AT-II—S100b PPC (60-80 min). Under these conditions the duration of drinking behavior was 15-20 min. The volume of water intake in these rats was much lower than in animals receiving free or BSA-bound AT-II (Fig. 1).

Combined treatment with the test substances and Capoten or saralasin abolished the dipsogenic effect of free AT-II. However, this treatment potentiated the dipsogenic effect of AT-II—BSA PPC. Induction of drinking behavior by AT-II—S100b PPC remained unchanged under these conditions.

Analysis of peripheral effects of free and protein-bound AT-II showed that the procedure of injection by itself was followed by a slight increase in SBP and HR in control animals (by 5-8% over 1 h). These data were taken into account in studying the variations of hemodynamic parameters in treated rats. PPC of AT-II and S100b had a hypertensive effect. The observed changes had a lower amplitude, but persisted for a longer period of time (compared to native AT-II; Fig. 2, *a*). Single injection of PPC of AT-II and S100b was followed by a significant increase in SBP (by 20-25 and 15-17% compared to the baseline value and physiological saline, respectively). This effect was observed by the 10th minute postinjection and persisted for 1 h.

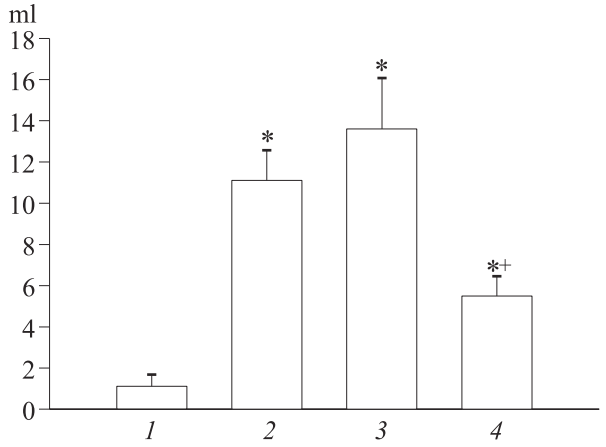

Fig. 1. Dipsogenic effects of free and protein-bound AT-II in non-thirsty animals. Ordinate: volume of water intake over 1.5 h after intraperitoneal injection. Control (*1*); AT-II (*2*); AT-II+BSA (*3*); and AT-II+S100b. p<0.001: *compared to the control (0.9% NaCl); +compared to free AT-II.

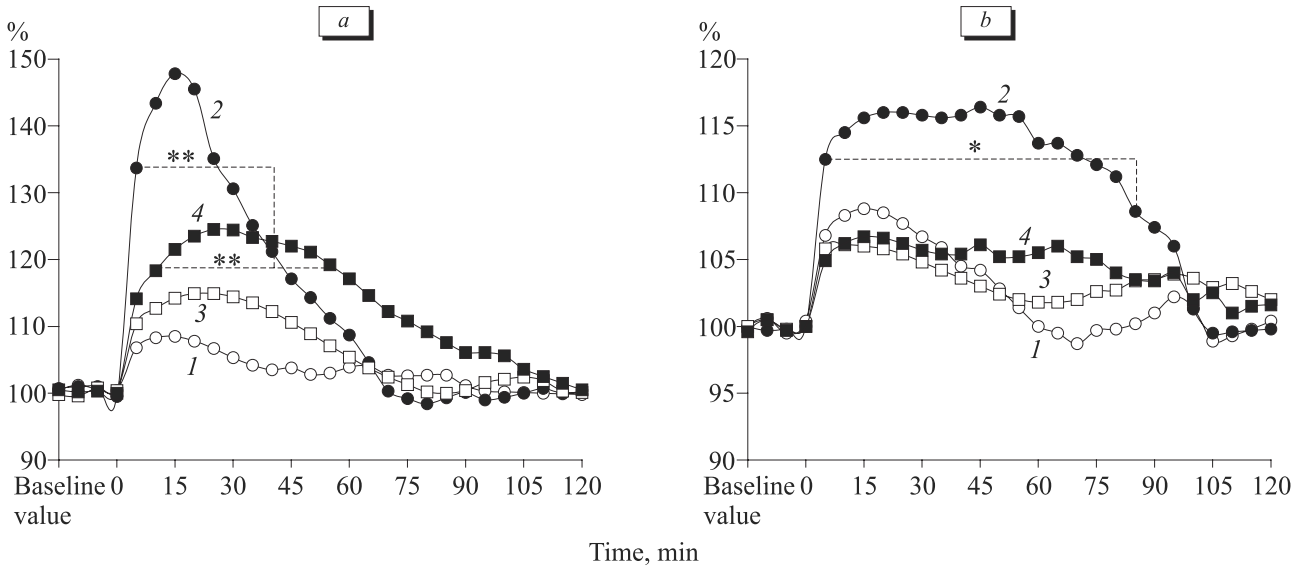

Fig. 2. Variations in SBP (*a*) and HR (*b*) after intraperitoneal injection of free and protein bound AT-II. Control (*1*); AT-II (*2*); AT-II+BSA (*3*); and AT-II+S100b. Straight lines: time intervals for significant differences between test parameters. *$p<0.001$ and **$p<0.01$ compared to the control.

The hypertensive effect of free AT-II manifested in a 48% increase in SBP. However, significant differences from the physiological saline group were revealed only during a 40-min period. As differentiated from the AT-II—S100b complex, hypertensive activity of PPC of AT-II and BSA was low (up to 15% of the baseline value) and did not differ from that of physiological saline.

Binding of AT-II to proteins was followed by inhibition of the positive chronotropic effect. Injection of native AT-II was accompanied by tachycardia (increase in HR by 16% of the baseline value), which persisted for 1.5 h. HR in animals receiving PPC increased by 6-7% of the baseline value. However, variations in HR after PPC injection did not differ from those induced by physiological saline (Fig. 2, *b*).

We studied possible mechanisms of physiological activity of synthetic complexes of AT-II and functionally different proteins. Administration of direct antagonist of AT_1 receptors losartan and competitive peptide antagonist of angiotensin AT_1 receptors saralasin in combination with free AT-II or AT-II—S100b PPC completely abolished the effects of the test substances. Angiotensin-converting enzyme inhibitor Capoten produced the same effect on SBP and HR variations induced by protein-bound AT-II. The hypertensive effect of free AT-II persisted, but became less significant after administration of Capoten.

Our results suggest that AT-II is differentially involved in the regulation of physiological functions, which results from complex formation of this peptide and functionally different proteins. These proteins modulate the interaction of AT-II with specific receptors, which determines the features, direction, and duration of physiological processes. This mechanism probably underlies the involvement of AT-II-protein complexes in adaptive and compensatory functions the organism.

It was hypothesized that physiological activity of AT-II in binding to specific receptors is mediated by different processes of intracellular signal transduction [3,5,6]. They provide a specific regulatory response of the cellular genome. Complexes of AT-II and functionally different proteins probably contribute to divergence of signal transduction pathways, which provides an adequate cell response with the involvement of the renin-angiotensin system.

Hence, the protein-peptide complex of AT-II and Ca^{2+}-binding protein S100b is primarily involved in the regulation of hemodynamics. However, the complex of angiotensin II and transport protein BSA plays a role in the formation and realization of drinking behavior.

REFERENCES

1. A. V. Kotov, S. M. Tolpygo, E. I. Pevtsova, and M. F. Obukhova, *Vestn. Ros. Akad. Med. Nauk*, No. 4, 36-43 (2001).
2. J. L. Aldons, *Nature*, **407**, 233-241 (2000).
3. D. Daniels, D. K. Yee, L. F. Faulconbridge, and S. J. Fluharty, *Endocrinology*, **146**, No. 12, 5552-5560 (2005).
4. B. Farsak, S. Gunaydin, C. Yorgancioglu, and Y. Zorlutuna, *Cardiovasc. Surg. (Torino)*, **44**, No. 1, 31-35 (2003).
5. L. Hunyady and K. J. Catt, *Mol. Endocrinol*., **20**, No. 5, 953-970 (2006).

6. M. Kurdi, W. C. De Mello, and G. W. Booz, *Intern. J. Biochem. Cell Biol.*, **37**, No. 7, 1357-1367 (2005).
7. T. Mello e Souza, A. Rohden, M. Meinhardt, *et al*., *Physiol. Behav*., **71**, No. 1-2, 29-33 (2000).
8. D. Menta and A. B. Malik, *Physiol. Rev*., **86**, 279-367 (2006).
9. H. Nishiyama, T. Knopfel, S. Endo, and S. Itohara, *Proc. Natl. Acad. Sci. USA*, **99**, No. 6, 4037-4042 (2002).
10. E. Savaskan, *Curr. Alzheim. Res*., **2**, No. 1, 29-35 (2005).
11. M. L. Schroeter, H. Abdul-Khaliq, A. Diefenbacher, and I. E. Blasig, *Neuroreport*, **13**, No. 13, 1675-1688 (2002).
12. B. S. Shastry, *Neurochem. Int*., **43**, No. 1, 1-7 (2003).
13. V. V. Sherstnev, M. A. Gruden', Z. I. Storozheva, and A. T. Proshin, *Neurosci. Behav. Physiol*., **33**, No. 1, 31-38 (2003).
14. M. Stoppini, A. Andreola, G. Foresti, and V. Bellotti, *Pharmacol. Res*., **50**, No. 4, 419-431 (2004).
15. S. J. Veerasingham and M. K. Raizada, *Br. J. Pharmacol*., **139**, No. 2, 191-202 (2003).