Complexes of Angiotensin IV with Functionally Different Proteins in the Regulation of Drinking Behavior and Hemodynamics in Rats

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We compared physiological activity of synthetic complexes from angiotensin IV and functionally different proteins (transport protein, bovine serum albumin; and neurospecific Ca^{2+}-binding protein, S100b) as model analogues of endogenous protein-peptide complexes. Physiological activity of angiotensin IV was specifically modified by these proteins. Our results suggest that complexes of angiotensin IV with bovine serum albumin and S100b are strong factors for the integration of central and peripheral functions at the homeostatic and behavioral level.

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

We previously studied the involvement of individual peptide components of the renin—angiotensin system into the central and peripheral mechanisms of regulation of autonomic functions and evaluated the role of these components in the development and performance of behavioral reactions, including the acquired targeted behavioral response [2,3]. Differences were found in the spectra of activities of free and protein-bound angiotensins. It was hypothesized that protein-peptide complexes (PPC) play a specific role in the intrasystemic and intersystemic integration of functions [2,3]. Our experiments showed that these complexes exhibit physiological activity, which is modified by functionally different proteins [4]. For example, "chimeric" compounds serve as the factors for prolonged maintenance of certain functional states and reproduction of the relevant behavioral skills. For instance, administration of PPC consisting of the major effector peptide of the renin—angiotensin system (angiotensin II, AT-II) and transport protein (bovine serum albumin, BSA) to rats causes thirst followed by the realization of operant drinking behavior. PPC of AT-II and neurospecific protein S100b mainly affects the hemodynamics (systolic blood pressure, SBP; and heart rate, HR), but does not modulate drinking behavior [4].

AT-II is synthesized during enzymatic processing of the angiotensin precursor protein and is cleaved by various enzymes (*e.g.*, aminopeptidases A and N, carboxypeptidase P, and angiotensin-converting enzyme) with the formation of a wide spectrum of end-products (fragment of $AT\text{-}II_{1\text{-}7}$, AT-III, AT-IV, *etc.*). Little is known about physiological activity of these peptides [7,9,11,15]. It is known that AT-IV preferentially binds to specific receptors (AT_4), but can exhibit affinity for AT_1 receptors and AT_2 receptors for AT-II. Activity of AT-IV is manifested in the neurotrophic effect and modulation of learning (model of defensive behavior). The results of previous studies suggest that AT-IV is involved in memory fixation at the molecular genetic level under various pathological conditions (atherosclerosis, hypertension, and memory disorders) [5,9,11-14].

Here we compared the role of free AT-IV and AT-IV bound to functionally different proteins (transport

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protein BSA and neurospecific Ca^{2+}-binding protein S100b) in the development and realization of complex acquired drinking behavior in rats. We evaluated the effect of complexes from AT-IV on hemodynamic parameters in animals (SBP and HR).

MATERIALS AND METHODS

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

Synthetic conjugates of AT-IV (American peptides) with BSA and S100b protein (Sigma) were synthesized in our laboratory. These compounds serve as model analogues of endogenous PPC. PPC of AT-IV and carrier protein molecules were synthesized using a bifunctional coupling agent 1-cyclohexyl-3(2-morpholine-ethyl)-carbodiimide (Sigma). Chromatography showed that these conjugates contain 8-10 and 5-7 molecules of AT-IV per molecule of BSA and S100b, respectively.

Physiological activity of AT-IV—BSA and AT-IV—S100b complexes was compared with that of native AT-IV. 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-IV (400 μg/kg), PPC of AT-IV and BSA, and PPC of AT-IV and S-100b. The doses of PPC were equivalent to 400 μg/kg AT-IV. Control rats received intraperitoneal injections of the test substances in combination with angiotensin-converting enzyme inhibitor Capoten (n=8) and direct antagonist of AT_1 receptors losartan (n=10) in a dose of 300 μg/kg. Activity of the test substances was compared with that of 0.9% NaCl (solvent).

The effects of PPC consisting of AT-IV and proteins on drinking behavior were studied on untrained rats maintained under conditions of free access to water (the volume of water intake in these rats was measured over 3 h after injection) and on animas during training of complex targeted drinking behavior in an automatic device. Skill performance was estimated in the follow-up period. This approach allowed us to evaluate the time characteristics and dynamics of successive stages of complex drinking behavior ("start" compartment – running to the "manipulative" compartment – "manipulation" with a disk (drinking bowl) – water drinking from a bowl – return to the "start" compartment) [1]. Hemodynamic parameters were studied by the indirect method with a NIBP system (AD Instruments). Awake rats were placed in plastic cages. Due to variability in baseline SBP and HR, changes in these parameters were expressed in percents of the initial value.

The results were analyzed by Student's t test.

RESULTS

AT-IV in PPC with functionally different proteins was shown to have various physiological properties. These differences were found at the central and peripheral level.

As differentiated from free AT-II and BSA-bound AT-II, administration of native AT-IV (n=8), PPC of AT-IV and BSA (n=8), and PPC of AT-IV and S-100b (n=8) was not accompanied by the induction of water intake in animals that had free access to water. Free AT-IV significantly reduced the performance of drinking behavior in rats that were trained in a complex drinking skill (n=8, p<0.01). The incidence of drinking reactions tended to increase after treatment with PPC of AT-IV and BSA (n=9; Fig. 1, *a*). PPC of AT-IV and S100b had no effect on the performance of acquired drinking behavior in animals (n=10; Fig. 1, *a*). This PPC did not modulate the process of training in a new skill for the reinforcement in an automatic device (change in the direction of disk movement to obtain water). As distinct from PPC of AT-IV and S100b, PPC of AT-IV-BSA and free AT-IV significantly suppressed the acquisition of this skill for water reinforcement (Fig. 1, *b*).

We evaluated peripheral effects of free AT-IV and protein-bound AT-IV. Injection of 0.9% NaCl to control animals (n=12) was followed by an insignificant increase in BP and HR (by 5-8%), which persisted for 1 h. This fact was taken into account when evaluating the significance of hemodynamic shifts in experimental animals after injection of the test substances. PPC of AT-IV and S100b (n=10) had a strong hypertensive effect (by the amplitude and duration; Fig. 2, *a*). Single injection of this PPC was followed by a significant increase in the mean SBP (p<0.05 compared to control animals receiving 0.9% NaCl). The observed changes persisted for 1.5 h. PPC of AT-IC and BSA (n=9) produced insignificant effects comparable with those of 0.9% NaCl. There are conflicting data on the action of free AT-IV on SBP. It should be emphasized that previous studies were mainly performed on experimental models of isolated blood vessels (vasodilation and vasoconstriction) [5,10-13]. Our experiments on awake animals (n=10) demonstrated the hypotensive effect of this peptide. The mean SBP in treated rats decreased by 13% (p<0.05 compared to control animals receiving 0.9% NaCl; Fig. 2, *a*).

The most significant changes in HR (effect and duration) were observed after treatment with PPC of AT-IV and S100b (p<0.05; Fig. 2, *b*).

Capoten and losartan abolished the effect of PPC of AT-IV-S100b and free AT-IV on hemodynamic parameters.

Hence, activity of AT-IV (one of the final products of angiotensinogen processing) differs from that of AT-

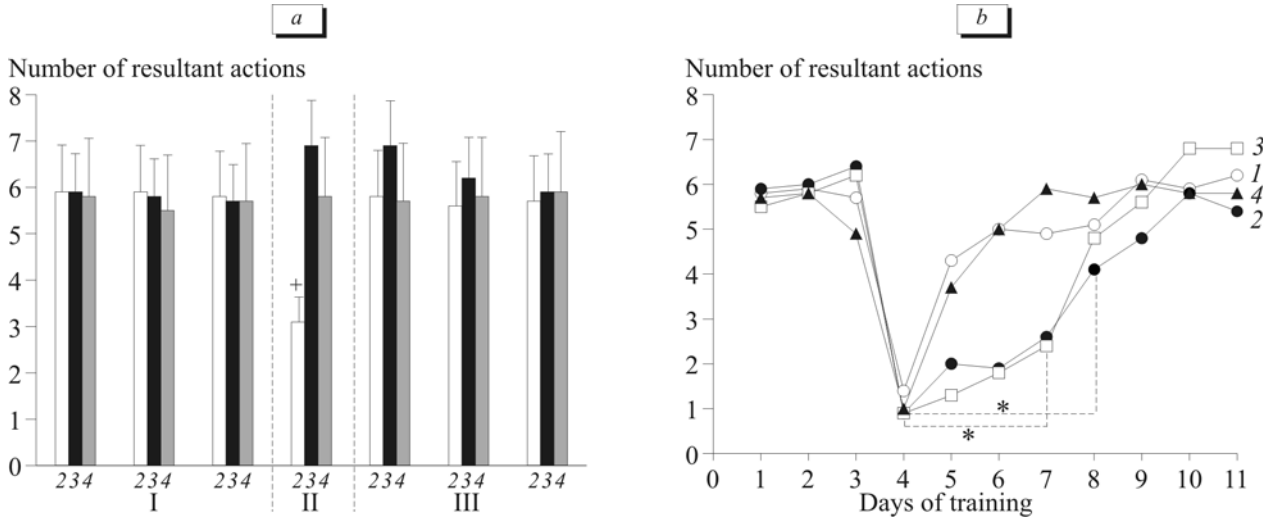

Fig. 1. Effects of free AT-IV and protein-bound AT-IV on the performance (*a*) and development (*b*) of operant drinking behavior in rats. Here and in Fig. 2: control, 0.9% NaCl (*1*); free AT-IV (*2*); AT-IV and BSA (*3*); AT-IV and S100b (*4*). Baseline value (I); administration of substances (II); aftereffect (III). Dotted line: time for the persistence of significant differences between test parameters. **p*<0.05 compared to the control; +*p*<0.01 compared to the baseline value.

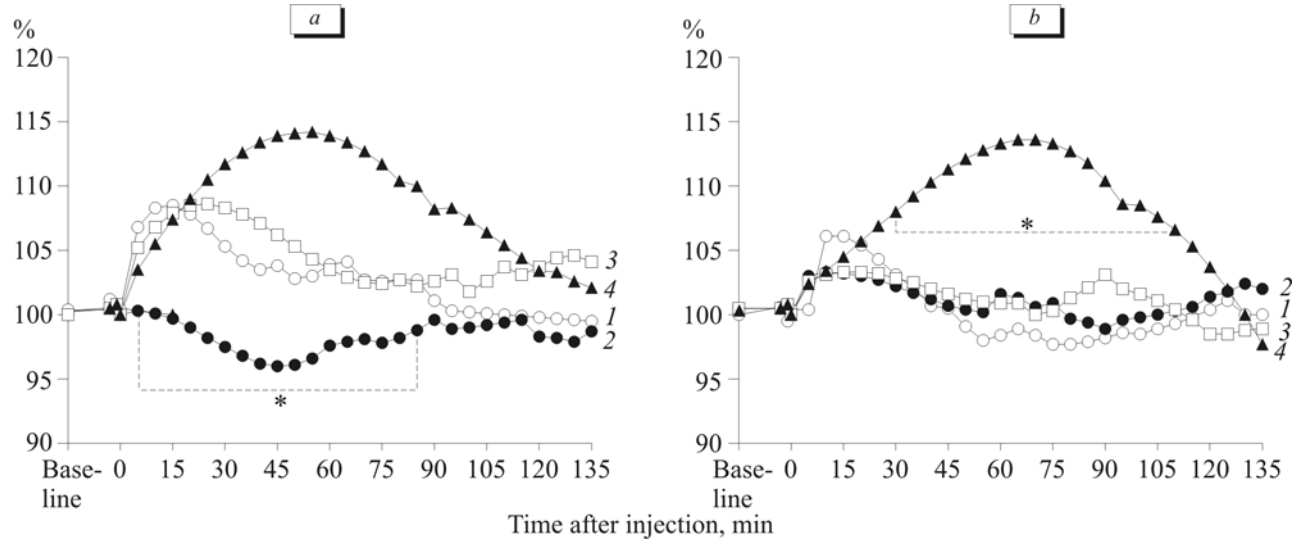

Fig. 2. Changes in SBP (*a*) and HR (*b*) after intraperitoneal injection of free AT-IV and protein-bound AT-IV.

II (the main effector peptide of the renin—angiotensin system). For example, free AT-IV (not bound to the protein) produces a strong hypotensive effect and suppresses the development and realization of complex acquired drinking reactions, but AT-IV does not induce the inherited drinking behavior.

We showed that activity of peptide is modulated by functionally different proteins. These data are consistent with the results of previous studies [4]. The complex of AT-IV and BSA mainly affects drinking behavior, while the complex of AT-IV and S100b has a strong influence on hemodynamic parameters. For example, PPC of AT-IV and BSA has a moderate activating effect on acquired behavior, suppresses the development of new drinking skills, and does not affect SPB and HR. The complex of AT-IV and S100b has other properties: induces sustained increase in SBP and HR, but has no effect on the performance of acquired drinking behavior and development of new skills.

It can be hypothesized that physiological activity of angiotensins during the interaction with various types of specific receptors is mediated by heterogeneous processes of signal transduction [6,8,9,15]. These changes contribute to a specific regulatory response, which involves the cell genome. Complexes of angiotensins with functionally different proteins determine the divergence of signal transduction pathways, which induces an adequate cell response with the involvement of the renin—angiotensin system (in the whole organism).

Various proteins (transport protein BSA and neurotrophic protein S100b) modulate activity of angiotensins (AT-II and AT-IV), which probably contributes to the cooperation or antagonism between some components of the renin—angiotensin system. These features are of considerable importance in the central and peripheral mechanisms of intrasystemic and intersystemic integration of functions.

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REFERENCES

1. V. S. Varlakov, A. P. Shcherbina, A. V. Shvadchenko, *et al*., *Automatic Device for Studying the Operant Behavior of Animals* [in Russian], Investor's Certificate SU No. 1813382 A 1 (1992).
2. A. V. Kotov, S. M. Tolpygo, E. I. Pevtsova, and M. F. Obukhova, *Vestn. Ros. Akad. Med. Nauk*, No. 4, 36-43 (2001).
3. A. V. Kotov, S. M. Tolpygo, E. I. Pevtsova, and M. F. Obukhova, *Ros. Fiziol. Zh*., **88**, No. 6, 686-698 (2002).
4. A. V. Kotov, S. M. Tolpygo, E. I. Pevtsova, and M. F. Obukhova, *Byull. Eksp. Biol. Med*., **146**, No. 8, 135-137 (2008).
5. S. Y. Chai, R. Fernando, G. Peck, *et al*., *Cell. Mol. Life Sci*., **61**, No. 21, 2728-2737 (2004).
6. D. Daniels, D. K. Yee, L. F. Faulconbridge, and S. J. Fluharty, *Endocrinology*, **146**, No. 12, 5552-5560 (2005).
7. M. de Casparo, K. J. Catt, T. Inagami, *et al*., *Pharmacol. Rev*., **52**, No. 3, 415-472 (2000).
8. L. Hunyady and K. J. Catt, *Mol. Endocrinol*., **20**, No. 5, 953-970 (2006).
9. M. Kurdi, W. C. De Mello, and G. W. Booz, *Int. J. Biochem. Cell Biol.*, **37**, No. 7, 1357-1367 (2005).
10. L. Loufrani, D. Henrion, D. Chansel, *et al*., *J. Pharmacol. Exp. Ther*., **291**, No. 2, 583-588 (1999).
11. T. Mustafa, J. H. Lee, S. Y. Chai, *et al*., *J. Renin Angiotensin Aldosterone Syst*., **2**, No. 4, 205-210 (2001).
12. M. R. Rosen and I. S. Cohen, *J. Physiol*., **570**, Pt. 2, 209-218 (2006).
13. M. Ruiz-Ortega, V. Esteban, and J. Egido, *Trends Cardiovasc. Med*., **17**, No. 1, 19-25 (2007).
14. B. Stragier, D. De Bundel, S. Sarre, *et al*., *Heart Fail. Rev*., **13**, No. 3, 321-337 (2008).
15. J. W. Wright, B. J. Yamamoto, and J. W. Harding, *Prog. Neurobiol*., **84**, No. 2, 157-181 (2008).