Hypotensive Effect of Potentiated Antibodies to Angiotensin II and AT_1 Receptors

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Hypotensive activity of ultralow doses of antibodies to angiotensin II and its receptors was studied on adult NISAG rats with hereditary stress-induced arterial hypertension. Antibodies to C-terminal fragment of angiotensin II receptors produced the most pronounced hypotensive effect, which was reproducible after repeated administration. These antibodies decreased systolic blood pressure by 16.40±0.62 mm Hg. The most rapid hypotensive effect was produced by affinity purified antibodies to angiotensin II: 2 h after administration of these antibodies systolic blood pressure decreased by 12.80±5.49 mm Hg. Our results indicate that combination treatment with ultralow doses of antibodies to angiotensin II and its receptors hold much promise for the use in clinical practice.

Key Words: *ultralow doses; antibodies; arterial hypertension; angiotensin II; angiotensin II receptors*

Angiotensin II (ATII) plays a central role in the regulation of systemic blood pressure (BP). This compound acts as a direct regulator and is involved in functional activity of the renin-aldosterone system. ATII produces a potent vasoconstrictor effect on vascular smooth muscles. The effects of ATII on myocardial contractility and heart rate are realized via the sympathetic nervous system. ATII modulates renal retention of sodium and water, stimulates synthesis and secretion of aldosterone, causes thirst, and initiates production of antidiuretic hormone. These data indicate that ATII produces systemic effects and, therefore, plays a central role in acute and chronic regulation of BP. Moreover, ATII produced in tissues affects the stricture and functions of vessels via its paracrine and autocrine effects. This alternative pathway of ATII production is catalyzed by some tissue enzymes. Local secretion of ATII determines remodeling in the cardiovascular system. Constant exposure of the vascular wall to mechanical factors can stimulate the alternative pathway of ATII production. Similar processes in the myocardium and renal glomeruli can induce reconstruction of these target organs and change their functional activity. ATII increases the density and sensitivity of receptors to growth factors that affect proliferation of smooth muscle cells (fibroblast growth factor, transforming growth factor-b_1, platelet-derived growth factor, and insulin-like growth factor). Overexposure of the vascular wall to ATII contributes to the development of pathological changes in atherosclerosis (*e.g.*, growth and migration of smooth muscle cells). ATII activates macrophages, promotes platelet aggregation, stimulates plasminogen activator inhibitor-1, and causes endothelial dysfunction. ATII stimulates atherogenesis via suppression of apoptosis, stimulation of oxidative stress, activation of leukocyte adhesion and migration, and initiation of thrombosis. Inhibition of ATII synthesis with angiotensin-converting enzyme inhibitors attenuates progressive damage to vessels during diabetic nephropathy, cardiac insufficiency, and myocardial infarction.

These data indicate that ATII is a perfect target for pharmacological correction of acute and chronic regulation of systemic BP and structure and functions of the cardiovascular system [7,12].

Practically all known physiological effects of ATII are realized via type 1 receptors (rAT_1). Binding of ATII to rAT_1 induces conformational changes in receptor molecule and activation of intracellular signal transduction pathways [5]. Binding to the agonist is followed by receptor desensitization and internalization (similarly to other G protein-coupled receptors).

Stimulation of rAT_1 intensifies aldosterone secretion in the adrenal glands, stimulates the sympathetic nervous system, and initiates production of antidiu-

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retic hormone [8]. Activation of the renin-angiotensin system (RAS) results in BP rise and stimulation of practically all regulatory mechanisms (vasoconstriction, increase in circulating blood volume, and cardiac output). These mechanisms play an important role in the development of secondary arterial hypertension (AH) and essential hypertension.

RAS is the main target for pharmacotherapy of AH. Functional activity of this system is suppressed by β-adrenoblockers, angiotensin-converting enzyme inhibitors, and rAT_1 antagonists [7].

Here we studied the hypotensive effect of potentiated antibodies (PAB) to the major components of RAS (ATII and rAT_1). For evaluation of significance of some characteristics for realization of pharmacological activity (purity and antigenic specificity of antibodies), we used antibodies against ATII (whole antiserum and affinity purified antibodies) and polyclonal antibodies to N- and C-terminal fragments of rAT_1.

MATERIALS AND METHODS

Experiments were performed on male NISAG rats aged 5-6 months [9]. The animals were kept in a vivarium at the Institute of Cytology and Genetics and had free access to water and food (fixed NaCl content). Ten days before the experiment, the rats were placed into individual cages and subjected to daily handling to prevent stress associated with this procedure and masking the effect of preparations.

The animals were divided into 5 groups (10 rats per each). Test preparations or vehicle were given perorally in a daily dose of 0.5 ml through a glass pipette before feeding (9.00-10.00).

Control rats received distilled water for 5 days. Systolic BP (SBP) was measured on days 1 and 5. Group 1 animals received homeopathically potentiated antibodies to ATII (PAB-ATII, whole antiserum) for 7 days. SBP was measured on days 1 and 7 and 7 days after administration of the last dose. Group 2 rats received affinity purified PAB-ATII for 5 days. SBP was measured on days 1 and 5. Group 3 animals received PAB to N-terminal fragment of rAT_1 (PAB-N-rAT_1) for 12 days and after a 8-day interval again for 5 days. SBP was measured on days 1, 5, and 12, 7 days after the first course, and 5 days after the second course of treatment. Group 4 rats received PAB to the C-terminal fragment of rAT_1 (PAB-C-rAT_1) for 5 days and after a 8-day interval again for 5 days. SBP was measured on days 1 and 5, 7 days after the first course, and 5 days after the second course of treatment.

The equivalent concentration of test preparations was 10^{-24} wt %.

SBP was measured on the tail by sphygmography 2-3 h after the last treatment under Rausch narcosis to exclude the effect of psychological stress on SBP.

The results were analyzed by Student's *t* test.

RESULTS

SBP significantly decreased 2 h after the 1st treatment with preparations. Therefore, the effect of PAB developed as rapidly as that observed after administration of modern hypotensive drugs. The course of treatment with both forms of PAB-ATII produced similar hypotensive effects, but rapid changes were observed only after administration of affinity purified antibodies, which confirmed the importance of removal of plasma factors and antibodies with another specificity. The revealed differences can be explained by previously described reaction to novelty [2]. After administration of affinity purified antibodies, this nonspecific reaction has a pronounced and more specific hypotensive component. This specific response disappears during primary reaction to ultralow doses of a mixture containing various substances.

Treatment with PAB-C-rAT_1 produced a more pronounced hypotensive effect than PAB-N-rAT_1 (Table 1). However, the effect of PAB-N-rAT_1 increased after long-term administration.

The N-terminal fragment of rAT_1 is responsible for binding of peptide ligands, including ATII. Non-

TABLE 1. SBP in NISAG Rats after Peroral Administration of PAB-ATII and PAB-rAT_1 (mm Hg, $X \pm m$, n=10)

Experimental conditions		Baseline SBP	ΔSBP	
			2-3 h after the 1st treatment	after administration for 5 (7^+) days
Control (water)$^+$		178.5±4.1	-5.0±3.3	2.9±4.1
PAB-ATII	whole antiserum$^+$	174.5±2.3	-2.8±3.1	-12.6±3.4**
	affinity purified	187.9±4.2	-12.8±5.5**	-12.6±8.7**
PAB-N-rAT_1		181.7±3.5	-3.9±3.1	-10.1±3.5***
PAB-C-rAT_1		180.0±1.9	-3.0±3.2	-16.0±8.9*

Note. *p<0.001, **p<0.01, and ***p<0.05 compared to the baseline level.

peptide rAT_1 antagonists bind to transmembrane domains of the receptor corresponding to binding sites in G proteins. The C-terminal fragment of rAT_1 plays a role in stimulation of tyrosine kinases and phospholipase Cg mediating the effects of ATII (similarly to growth factors) [5]. This fragment is involved in receptor internalization, an important step in the regulation of activity of the receptor cycle.

Seven days after withdrawal of test preparations SBP in rats of groups 3 and 4 returned to the baseline level. Interestingly, the hypotensive effect of PAB-C-rAT_1 was highly reproducible after repeated administration. In group 3 and 4 rats SBP decreased by 13.1±4.1 ($p<0.01$) and 16.4±0.6 mm Hg ($p<0.001$), respectively, on day 5 of repeated treatment. This reproducibility of the effect of PAB- rAT_1 and PAB-C-rAT_1 unambiguously attest to their hypotensive activity. In our experiments the regulatory effect on peripheral components of RAS was most effective.

It should be emphasized that apart from rAT_1, a more rare type of ATII receptors, rAT_2, was identified. Stimulation of rAT_2 produces opposite changes [5]. This can explain different effects of PAB-ATII and PAB-rAT_1. However, this problem requires further detailed investigations.

Despite obvious analogy with synthetic antagonists of ATII receptors PAB-rAT_1 belong to a principally new class of preparations containing potentiated antibodies and characterized by clinical effectiveness and safety [3].

The biological effects of PAB can be associated with changes in the system of natural antibodies possessing regulatory activity [14]. Our previous studies showed that PAB affect production of immune antibodies [1].

It is unlikely that experiments on complex biological models will elucidate the mechanism of primary changes in biological systems induced by peroral administration of antibodies in ultralow doses, but new physical and chemical properties accepted by the substance during homeopathic potentiation should be evaluated.

Our results indicate that PAB-ATII and PAB-rAT_1 possess pronounced hypotensive activity. The effects of combination treatment with PAB-ATII and PAB-rAT_1 in ultralow doses are of particular interest. Test PAB produced a hypotensive effect in animals with stress-induced AH, which indicates that these preparations hold much promise for pathogenetic therapy of hypertension of different severity.

REFERENCES

1. V. I. Agafonov, N. V. Bel'skaya, M. G. Danilets, *et al*., *Byull. Eksp. Biol. Med.*, Suppl. 5 (2002).
2. T. M. Vorob'eva, *Novelty as a Stimulatory Factor during Treatment with Pharmacological Preparations*, Abstract of Cand. Med. Sci. Dissertation, Kharkov (1962).
3. M. B. Shtark, T. M. Vorob'eva, O. I. Epshtein, *et al*., *Sib. Vestn. Psikhiatr. Narkol.*, **21**, No. 3, 111-113 (2001).
4. M. Boes, C. Esau, M. B. Fischer, *et al*., *J. Immunol*., **160**, 4776-4787 (1998).
5. M. De Gasparo, K. J. Catt, T. Inagami, *et al*., *Pharmacol. Rev*., **52**, 415-472 (2000).
6. S. M. Gardiner, T. R. Auton, M. R. Downham, *et al*., *Br. J. Pharmacol*., **129**, No. 6, 1178-1182 (2000).
7. E. J. Jacobson, *Geriatrics*, **56**, No. 2, 20-28 (2001).
8. S. Julius and S. Nesbitt, *The ABCs of Antihypertensive Therapy*, Ed. F. H. Messerli, Lippincott, New York (2000), pp. 13-24.
9. A. L. Markel, *Genetic Hypertension*, Ed. J. Sassard, L. (1992), **28**, pp. 405-407.
10. F. H. Messerli, E. Grossman, M. Weber, *et al*., *The ABCs of Antihypertensive Therapy*, Ed. F. H. Messerli, Lippincott, New York (2000), pp. 124-147.
11. J. B. Michel, F. X. Galen, C. Guettier, *et al*., *J. Hypertens. Suppl*., **7**, No. 2, S63-S70 (1989).
12. M. R. Weir and V. J. Dzau, *Am. J. Hypertens*., **12**, No. 12, Pt. 3, 205S-213S (1999).