Hypotensive Activity of Ultralow Doses of Antibodies to Factors Involved in the Regulation of Vascular Tone

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Hypotensive activity of ultralow doses of antibodies to some endogenous substances involved in the regulation of vascular tone was studied on NISAG rats with hereditary stress-induced arterial hypertension. It was found that antibodies to angiotensin II and its receptor in ultralow doses markedly reduced systolic blood pressure, which was reproducible after repeated treatment. The course of peroral treatment with antibodies to endothelin and endothelial NO synthase in ultralow doses did not decrease systolic blood pressure.

Key Words: *antibodies; ultralow doses; arterial hypertension; pharmacological screening; angiotensin*

Rational pharmacotherapy of arterial hypertension remains an urgent problem because of high incidence of cardiovascular diseases, disability, and mortality from essential hypertension and symptomatic arterial hypertension. There are no hypotensive drugs normalizing blood pressure and not causing side effects that decrease the quality of life in patients. The search for new potent hypotensive preparations is especially important in Russia, where economic factors limit the use of these drugs.

In antihypertensive therapy, preference is given to substances that specifically modulate the renin-angiotensin system playing a key role in the regulation of vascular tone [4]. These preparations include inhibitors of angiotensin-converting enzyme and antagonists of angiotensin II receptors. The effects of these preparations are related to binding and inhibition of target molecules with ligands.

Previous experiments performed at the Materia Medica showed that ultralow doses of antibodies (AB) to endogenous regulator (antigen) obtained by homeopathic potentiation do not inactivate the regulator, but modify its molecule [2]. In light of this studies of the effects produced by potentiated AB during the therapy of arterial hypertension are of considerable interest. Here we performed primary screening for antihypertensive activity of potentiated AB to substances involved in the regulation of vascular tone and potentiated angiotensin I.

MATERIALS AND METHODS

Experiments were performed on NISAG rats with hereditary stress-induced arterial hypertension obtained after long-term selection at the Institute of Cytology and Genetics [5]. We used adult male rats with developed arterial hypertension aged 5-6 months. The animals were kept in a vivarium of the Institute of Cytology and Genetics and had free access to water and food with controlled NaCl content. Ten days before the experiment, the rats were placed into individual cages and daily handled to prevent stress associated with this procedure and masking the effect of preparations. Test preparations or potentiated distilled water (control) were given perorally in a daily dose of 0.5 ml through a glass pipette before feeding for 5 or 7 days (9.00-10.00). We studied the following potentiated antibodies: AB to angiotensin II (PAB-AII, whole antiserum or affinity purified), AB to N- and C-terminal fragments of angiotensin II receptor (PAB-N-RA and PAB-C-RA), AB to endothelin (PAB-E), AB to endothelial NO synthase (PAB-eNOS), and potentiated angiotensin I.

Test substances were administered as a mixture of homeopathic dilutions (C12+C30+C200) obtained by homeopathic potentiation. The relative equivalent concentration was not more than 10^{-24} wt %.

Systolic blood pressure (SBP) was measured on the tail by sphygmography 2-3 h after the last treatment under Rausch narcosis for preventing the effect of psychological stress on SBP.

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TABLE 1. Changes in SBP Produced by Potentiated AB Administered Perorally to NISAG Rats for 5 or 7 Days (mm Hg, $\bar{X}\pm m$, n=8-10)

Experimental conditions		Before administration	After administration
7-day treatment	vehicle (distilled water)	178.30±4.13	175.40±3.07
	PAB-AII (whole antiserum)	174.50±2.32	161.70±3.23**
5-day treatment	PAB-AII (affinity purified)	187.90±4.16	175.30±7.48**
	PAB-N-RA	181.70±3.49	171.6±4.3***
	PAB-C-RA	180.00±1.91	164.00±1.86*
	PAB-E	174.40±3.33	169.80±2.87
	PAB-eNOS	187.1±2.7	180.30±4.33
	angiotensin I	180.0±2.9	191.0±3.8***

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

RESULTS

ATA-II significantly decreased SBP in NISAG rats (Table 1). Differences in the variability of SBP after the course of treatment with affinity purified AB and potentiated antiserum were probably related to a shorter period of AB administration.

Antagonists of angiotensin II receptors mediating practically all effects of this peptide and playing a role in the pathogenesis of arterial hypertension are promising antihypertensive drugs preventing the development of complications [9]. PAB-N-RA and PAB-C-RA were most effective in our experiments (Table 1). Different effectiveness of these preparations probably reflects the role of different fragments of angiotensin receptor in the realization of its functional activity.

Endothelin is a potent vasoconstrictor peptide and tissue hormone produced in the vascular endothelium [3]. Peroral administration of PAB-E for 5 days did not significantly decrease SBP (Table 1), which casts some doubt on the significance of this compound as a hypotensive preparation. Published data also suggest that synthetic endothelin blockers do not produce positive effects during treatment of arterial hypertension. M. Stimpel reported that although highly specific antagonists of endothelin receptors are now available, the role of endothelin in the physiological regulation of systemic blood pressure remains unclear [8]. Endothelin most likely is involved in the regulation of local blood flow and modulates tone of vessels where it is produced.

NO is a physiological antagonist of endothelin in tissues. Local regulation of vascular tone by the endothelium is realized via NO production catalyzed by eNOS. Endothelial dysfunction accompanying various cardiovascular diseases is manifested in abnormal production of NO in the endothelium [6]. In our experiments peroral administration of PAB-eNOS for 5 days did not affect SBP (Table 1), which is consistent with modern notions on the role of NO in the regulation of tone in large and intermediate vessels, in particular, in response to mechanical strain of the vascular wall [7]. Probably, the contribution of these arteries in the total peripheral resistance is lower that the contribution of small vessels sensitive to pressor effect of angiotensin II. Previous studies showed that the endothelium plays a role in the adaptation to ischemia, and PAB-eNOS improve copulatory activity [1]. Therefore, the effects of PAB-eNOS should be studied on the model of local ischemia.

Administration of potentiated angiotensin I for 5 days significantly increased SBP (Table 1). Therefore, potentiated precursor of angiotensin II administered perorally in a dilution surpassing the Avogadro's number simulates the pressor effect of its molecules. These data confirm high biological activity of ultralow doses of homeopathically potentiated substances, on the one hand, and the possibility of using potentiated angiotensin I for the therapy of pathological conditions associated with hypotension.

Our results indicate that test substances possess moderate hypotensive activity, which prevents considerable variations in blood pressure causing undesirable effect (*e.g.*, impaired blood supply to the heart).

It should be emphasized that hypotensive activity of ultralow doses of substances prepared using homeopathic technique was studied on rats with stress-induced arterial hypertension. Undoubtedly, stress serves as a pathogenetic factor of hypertension. The efficiency of test substances under these conditions suggests that they hold promise for the therapy of essential arterial hypertension.

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