Inhibition of NO-Synthase Causes Stable Pressor Reaction under Conditions of 10-Minute Infusion of Angiotensin-2 to Narcotized Rats

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Infusion of angiotensin-2 (AT-2) is known to produce first an acute hypertensive reaction, followed by a drop of the mean arterial pressure (MAP). This response is described in the literature as tachyphylaxis [1] or the "escape phenomenon" [5]. The "escape" from the pressor influence of AT-2 may be assumed to be related to the system of endothelial relaxation factor (nitric oxide) generation, since NO synthesized from L-arginine under the influence of NO-synthase has been shown to be involved in the regulation of arterial pressure [2-4].

The aim of the present study was to evaluate the dynamics of MAP upon infusion of AT-2 in the presence and absence of N^G-monomethyl-L-arginine (N^G-MMLA), a specific competitive inhibitor of NO-synthase.

MATERIALS AND METHODS

Experiments were carried out on white outbred female rats weighing 180-230 g under nembutal narcosis (60 mg/kg, intraperitoneally). The animals breathed spontaneously through a tracheostomy tubing. The preparations were infused through the right jugular vein at a rate of 0.03 ml/min during 10 min. Pulse pressure and MAP were measured with an EMT-311 electromanometer (Mingograf-81, Elema-Shonander) on the left carotid artery. The experimental animals were divided into 6 groups and treated as follows: group 1 (n=7) - infusion of AT-2 (0.5 μg/kg/min); group 2 (n=8) - simultaneous infusion of AT-2 (0.5 μg/kg/min) and N^G-MMLA (1.25 mg/kg/min); group 3 (n=5) - simultaneous infusion of AT-2 (0.5 μg/kg/min), N^G-MMLA (1.25 mg/kg/min), and L-arginine (3 mg/kg/min); group 4 (n=7) - infusion of AT-2 (1.0 μg/kg/min); group 5 (n=7) - simultaneous infusion of AT-2 (1.0 μg/kg/min) and N^G-MMLA (1.25 mg/kg/min); group 6 (n=4) - infusion of N^G-MMLA (1.25 mg/kg/min).

The preparations used were hypertensin (Ciba), L-arginine (Reanal, Hungary), and N^G-MMLA, kindly supplied by Dr. S. Moncada (Wellcome Research Laboratories, UK)

The data were processed statistically using the Student t test.

RESULTS

Intravenous infusion of AT-2 in a dose of 0.5 μg/kg/min (1st group) induced a marked hypertensive reaction, followed by "escape" from the pressor influence of AT-2, so that after 10 min of infusion MAP differed little from the initial level (Table 1). Blockade of NO-synthase resulted in a

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TABLE 1. Dynamics of MAP and DE during 10-min Infusion of AT-2 under Conditions of Intact (Groups 1 and 4) and Blocked (Groups 2 and 5) NO-Synthase, and after Simultaneous Administration of Inhibitor and Substrate of the Enzyme (Group 3). (Group 6: Dynamics of MAP during 10-min Infusion of NO-Synthase Inhibitor)

Group	MAP, mm Hg				DE, %
	initial	2 min	5 min	10 min	
1 (n=7)	99.7±8.1	131.7±10.5*	122.2±10.5	112.8±9.1	62.7±7.6
2 (n=8)	103.3±2.6	138.0±1.7*	141.7±3.0*	141.3±2.4*	1.2±0.6
3 (n=5)	104.0±2.4	139.5±4.5*	135.5±4.9*	126.5±4.3*	40.3±8.8
4 (n=7)	111.0±3.4	159.3±3.8*	148.3±4.2*	140.1±3.9*	44.1±7.3
5 (n=7)	109.4±2.5	157.7±3.7*	163.4±3.8*	163.5±3.3*	3.0±1.6
6 (n=4)	99.3±3.7	108.7±1.9	109.7±2.6	110.0±2.3*	–

Note. *: $p<0.05$ in comparison with initial value in the corresponding group.

steadfast elevation of MAP after AP-2 infusion (2nd group), i.e., the "escape" phenomenon was not observed (Table 1). L-arginine added to the infusion mixture containing AT-2 and N^G-MMLA (3rd group) restored this phenomenon. The heart rate in the animals of the 1st-3rd groups remained unchanged under these conditions. N^G-MMLA in a dose of 1.25 mg/kg/min (6th group) produced a stable hypertensive reaction though much less pronounced than that in the 1st-3rd groups, the heart rate dropping in the 10th min of infusion from 292±25 to 266±26 beats/min.

Administration of a twofold dose of AT-2 both alone and together with N^G-MMLA (5th group) demonstrated that the dynamics of MAP was qualitatively similarly to that observed in the 1st and 2nd group (Table 1).

Thus, inhibition of NO-synthase qualitatively alters the pressor reaction in response to AT-2, the changes being most noticeable when determining the degree of "escape" (DE) from the pressor influence of AT-2 (Table 1). DE was calculated using the formula [5]:

$$\Delta E=\Delta P_1/\Delta P_2\times 100 \text{ (in \%)},$$

where ΔP_1 is the difference between maximal AT-2-induced MAP and that developed by the 10th min of infusion; ΔP_2 is the difference between maximal AT-2-induced and preinfusion MAPs. In the first group DE was 62.7±7.6%, whereas in the 2nd group it dropped to 1.2±0.6% ($p<0.001$). Consequently, the system of NO generation greatly contributes to the mechanisms of alleviation of the pressor response during a 10-min infusion of AT-2. Additional evidence is provided by the data obtained in rats of the 3rd group, in which DE was 40.3±8.8% vs. 1.2±0.6% in the 2nd group, $p<0.001$. DE in the 4th and 5th groups also differed considerably (44.1±7.3% and 3.0±1.6%, respectively). Thus, the "escape" from the pressor influence of AT-2 *in vivo* depends essentially on adequate functioning of the system of endothelial relaxation factor (NO) generation. It may be assumed that "shear stress" plays the most important role in enhanced NO generation in response to AT-2 infusion.

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