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Role of Extracellular Calcium in Endothelin-1-Induced Vasoconstriction

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In early studies of the mechanism of action of endothelin-1 (ET-1), a vascular endothelium-derived vasoconstrictor peptide, it was suggested that it produces an effect typical of an endogenous antagonist of voltage-dependent channels and that its activity as a vasoconstrictor depends on the concentration of extracellular Ca^{2+} and is suppressed in the presence of antagonists of these channels [10,11]. However, later this hypothesis was not accepted by all authorities. Discrepancies in the data obtained were due to determinations of the pathways of Ca^{2+} entry into the cell and of the intracellular pathways of signal transfer from receptor to effector (cell contractile proteins). In particular, it was thought [7] that the effect of ET-1 upon Ca^{2+} channels is not a direct one, but is mediated by the penetration of cations through nonselective ion channels and by membrane depolarization to the level critical for Ca^{2+} entry. The present paper is a continuation of our studies [1] of the mechanism of the ET-1 effect on vascular smooth muscle in rats.

In this study the role of extracellular Ca^{2+} ions in the contractile response was investigated, along with the pathways whereby they enter smooth muscle cells.

MATERIALS AND METHODS

The experiments were carried out on deendothelized smooth muscle preparations: helical strips of the thoracic aorta of Wistar rats. The contractility of the aorta strips was recorded with the aid of a DU-1 isometric force transducer on a Gemini recorder (Italy). The initial load was 1.5 g. The strips were placed in a thermostatically controlled chamber filled with normal oxygen-saturated Krebs solution of the following composition (mM): NaCl 130, KCl 4.6, $NaHCO_3$ 3.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, HEPES 10, and glucose 11, pH 7.4. Na-free Krebs solution was prepared by replacing NaCl with an equimolar amount of N-methyl-D-glucosamine (NMDG). Ca-free solution was prepared

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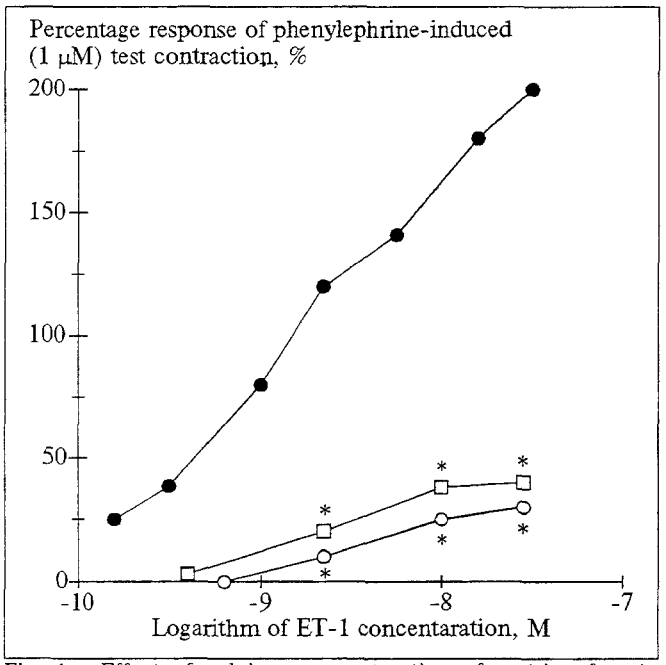

Fig. 1. Effect of calcium on contraction of a strip of aorta in the presence of ET−1. *1*) control; *2*) removal of Ca^{2+} from medium and addition of EGTA; *3*) in the presence of nifedipine (1 mM).Asterisk: $p<0.05$ vs. control.

by replacing all $CaCl_2$ with the corresponding amount of NaCl and adding 0.5-1 mM EGTA.

Phenylephrine, NMDG, nifedipine (Sigma), and endothelin (Nowa) were used in the study.

RESULTS

The relative contribution of extra- and intracellular calcium to the activation of contraction varies depending on the agonist, type of vessel, animal species, etc. As in our previous research [1], in the present work we observed no phasic component of the contractile response of the aorta to ET-1. According to data in the literature [7,9], this component of contractility is determined by the mobilization of intracellular calcium from the sarcoplasmic reticulum and its transfer via the nonselective ion channels and voltage-dependent channels. Since this component was not a major one, the main attention was paid to the participation of extracellular calcium in the mechanisms of contraction.

The role of extracellular Ca^{2+} ions in the generation of contraction of the smooth muscle cells can be established in experiments using Ca-free EGTA-containing solution. Under these conditions, ET-1 causes a contraction amounting to not more than 20% of the response for normal Krebs solution (Fig. 1). Hence, extracellular Ca^{2+} plays a crucial role in the development of ET-1-induced contraction of the smooth muscle cells of the aorta.

Since extracellular Ca^{2+} enters the cells mainly via the voltage-dependent and receptor-controlled channels [4,8], we tried to assess their relative contribution to the development of the contractile response. For this purpose we used nifedipine, an antagonist of the L-type voltage-dependent channels. A 15-min preincubation of a strip with 1 μM of nifedipine markedly affected the amplitude of contraction: the dose-effect curve shifted to the right-bottom, i.e., the minimum effective dose increased and the maximum response dropped 30% (Fig. 1). When nifedipine was added at the plateau of the ET-induced contractile response, this led to a rapid relaxation (by 40-60% of the attained level of contractility), following which the mechanical tension was maintained at a constant level.

Activation of the voltage-dependent Ca^{2+} channels requires membrane depolarization to the critical level [3]. What is the mechanism of such ET-1-induced depolarization? The results of our findings showed that ET-1-induced depolarization was caused by a sodium ion flux which probably travels through the receptor-controlled Na^+-permeable channels. This conclusion is based on the fact that in the solution where sodium ions were replaced

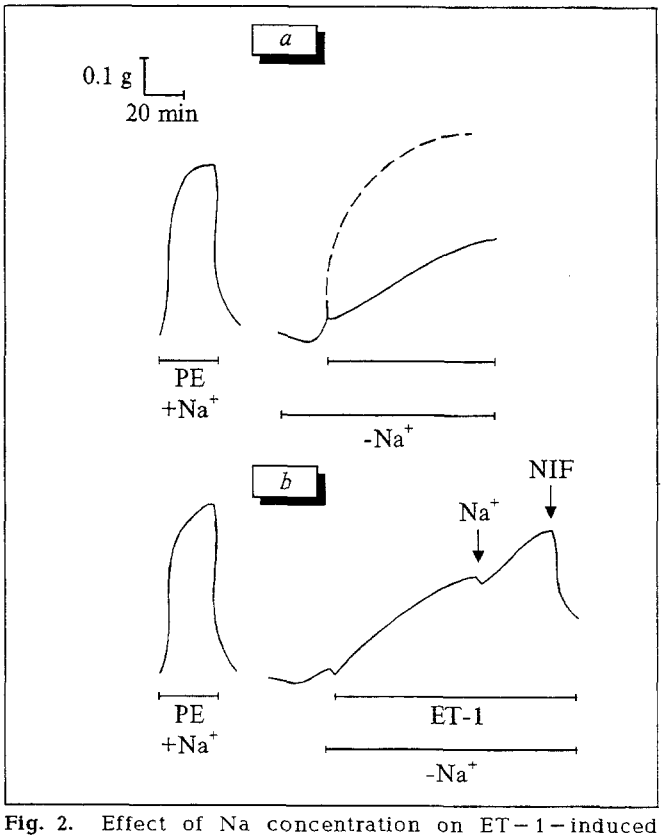

Fig. 2. Effect of Na concentration on ET−1−induced contraction of aorta. Left: test response to phenylephrine in normal solution. Right: replacing Na^+ with NMDG (*a*) and reverting to normal solution (*b*). Broken line: response induced by ET−1 in normal solution.

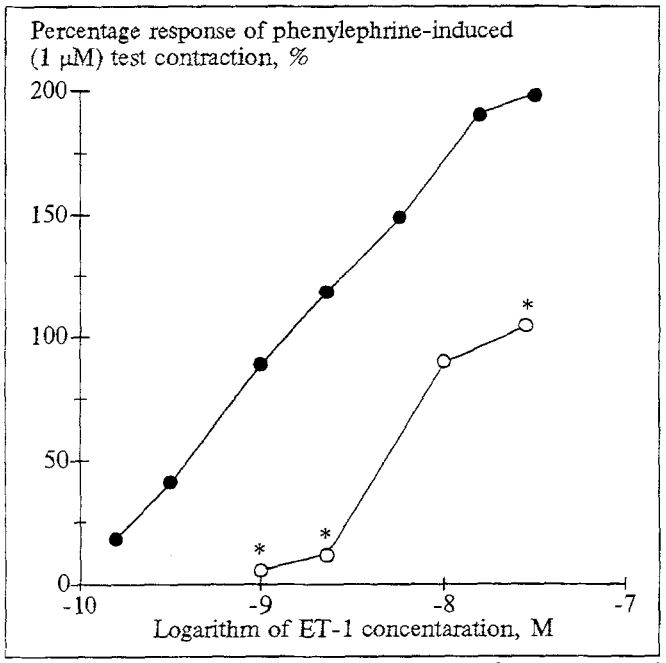

Fig. 3. Contraction of a strip of aorta in the presence of ET−1 in hyperpotassium solution. *1*) control contraction in normal solution; *2*) effect of ET−1. Asterisk indicates $p<0.01$ vs. control.

with NMDG, the amplitude of the contractile response to ET-1 proved to be markedly lower than that in the control (Fig. 2, *a*). It should be especially stressed that the addition of nifedipine at the plateau of ET-1-induced contraction did not alter the mechanical tension of the aorta strip in the NMDG-containing solution. In the Na-free solution sensitivity to nifedipine was observed when the NMDG-containing solution was replaced with normal Krebs solution at the plateau of ET-1-induced contraction (Fig. 2, *b*).

The results obtained seem to provide evidence that in Na-free solution the ET-1-induced membrane depolarization necessary to open the voltage-dependent Ca^{2+} channels is absent or reduced.

When the nifedipine-sensitive calcium entry is suppressed and sodium is replaced with NMDG, the nifedipine-resistant component of ET-1-induced contraction appears. ET-1 is capable of causing contraction of the aorta in Na^+- and Ca^{2+}-free medium; under such conditions the addition of Ca^{2+} ions (up to 1.5 mM) raised the mechanical tension by 50% of the amplitude of the response induced by 1 μM phenylephrine. These data attest to the presence of a nifedipine-resistant component of Ca^{2+} entry into the smooth muscle and to the participation of reserve Ca^{2+} in the generation of ET-1-induced contraction. The nifedipine-resistant component may appear due to the penetration of Ca^{2+} not via the voltage-dependent channels, but via the receptor-controlled channels.

One technique making it possible to study the receptor-dependent Ca^{2+} entry is the use of depolarized smooth muscle preparations [3]. In our experiments preliminary depolarization of the aorta preparation in the solution containing 130 mM KCl reduced the strength of the ET-1-induced contractile response by 50% (Fig. 3). The blocking of calcium channels with nifedipine against the background of a plateau response caused by endothelin in a depolarized strip of the aorta entirely relaxed the smooth muscle cells (Fig. 3). Thus, the nifedipine-resistant component of endothelin-induced contraction disappears for K depolarization. One may assume that under these conditions either Ca^{2+} entry not sensitive to nifedipine is absent, or the channels providing for its entry become more sensitive to nifedipine.

When considering the results of our findings, we should first of all note the participation of the L-type voltage-dependent Ca^{2+} channels in the generation of the ET-1-induced contractile response of the aorta, which has been confirmed by other studies [8,11]. Calcium delivered through these channels is able to provide for as much as 60% of tonic contraction (Fig. 1). According to our data, depolarization necessary to open the channels of this type is caused by the incoming flow of sodium ions (Fig. 2). In the literature we find other explanations of the mechanism of membrane depolarization in the presence of ET-1. In particular, the membrane voltage of the cell has been shown to depend on the activation of the Ca^{2+}-dependent Cl^- channel and on the exit of these ions from the cell [5]. At the same time, Na^+ possibly enters the cell along with Ca^{2+} [6]. It cannot be ruled out that discrepancies in the data are due to the object of investigation per se.

Following the suppression of nifedipine-sensitive Ca^{2+} entry, the nifedipine-resistant component of contraction responding to ET-1 was observed, which disappeared during K^+-induced depolarization of the cells. From published data [2] one may assume that ET-1 is capable of modulating the voltage-dependent calcium channels activated during hyperpotassium depolarization, this altering their sensitivity to nifedipine. Under these conditions the ET-1-induced contractility of the depolarized strip increases due to the increased Ca^{2+} entry via the nifedipine-sensitive voltage-dependent Ca^{2+} channels.

The results, when summarized, lead to the conclusion that the excitatory effect of ET-1 on the smooth muscle cells of the vessels studied is associated with the involvement of the Ca^{2+}-calmodulin-dependent pathway of signal transfer, which

is predominantly activated by the calcium supplied via the voltage-dependent and partially via the receptor-controlled channels.

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The Possibility of Using Glutathione as a Protector during Exposure to Hypoxia

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The disturbance of energy metabolism in the cell during hypoxia is primarily associated with restricted activity of the respiratory chain at the level of the 1st enzyme complex [2-5,8,9].

Recovery of the electron-transporting function of the NAD-dependent site of the respiratory chain may appreciably reduce the damage caused by hypoxia and raise the organism's resistance to oxygen deficiency. One of the possible methods of thus restoring the work of the respiratory chain is the use of artificial electron acceptors, which can directly transfer electrons from NADH to the 3rd enzyme complex, bypassing the blocked site [4,5,9,13].

Possible protective, antihypoxic properties of glutathione, a natural metabolite of the organism and an active acceptor of reduction equivalents, were studied in the present work.

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MATERIALS AND METHODS

The study was carried out on females and offspring of albino nonpedigree rats kept on a standard diet in the vivarium. Resistance to hypoxia was assessed in the offspring of rats administered an aqueous solution of reduced glutathione in a dose of 15 mg/kg intragastrically on days 16-19 of gestation. The time of survival at an "altitude" of 11,000 m (reserve time, RT), determined after Purshottam [16], served as the test of individual resistance to acute hypoxia.

The effect of glutathione on the hypoxic myocardium was studied on the model of the perfused heart. Animals with a low resistance to hypoxia

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