Effect of Endothelin-1 on the Adrenoreactivity of Blood Vessels and on the Participation of G-Proteins and Protein Kinase C in Endothelin-1-Induced Vasoconstriction

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Endothelin (ET) is a peptide produced by the cells of the vascular endothelium and is one of the most effective endogenous vasoconstrictive agents. The first reports on its isolation, purification, and synthesis appeared in 1987-88 [2]. In the interim it has been shown that in the mammalian organism at least three types of endothelin (ET-1, ET-2, and ET-3) are synthesized, of which only ET-1 is of endothelial origin. The vascular smooth muscles respond to ET-1 by a slowly developing strong contraction. ET-1 interacting with a receptor has been shown to cause, like other vasoconstrictors, activation of the phosphatidylinositol cycle, mobilization of intracellular calcium (Ca^{2+}), and activation of protein kinase C (PKC) [10,13]. However, there are also marked differences in the dynamics and pathways of the transfer of the intracellular signal [5,10]. Meanwhile, the study of the mechanism of action of ET-1 is not only of theoretical, but also of practical interest, since it has been suggested that ET directly participates in such pathological processes as the development of ischemic damage to the brain [6], changes in the neurosecretory processes, a reduction of afferent activity of the baroreceptors [12], and the development of arterial hypertension [4].

The aim of the present study was to investigate the kinetics of the contractile response of the rat aorta to ET-1 and the effect of ET-1 on the activity of α-adrenoreceptors of the smooth muscles in the caudal artery and to determine the role of membrane G-proteins and of PKC in the formation of ET-1-induced contraction in smooth muscle cells.

MATERIALS AND METHODS

The experiments were carried out on deendothelized smooth muscle preparations: helical strips of the thoracic aorta and segments (1 cm long) of the caudal artery of Wistar rats. The animals were killed by decapitation. The endothelium was removed by being air-blown (aorta) or by perfusion with distilled water (artery). The contraction of aorta strips was recorded with the aid of DU-1 isometric force transducers on a 2-channel Gemini registrator (Italy). The initial load was 1.5 g. The strips were placed in a thermostatically controlled chamber (37°C) filled with oxygen-saturated Krebs solution (pH 7.4) of the following content (mM): NaCl 130, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, HEPES 10, and glucose 11. The caudal artery was also placed in such a chamber and was fixed at both

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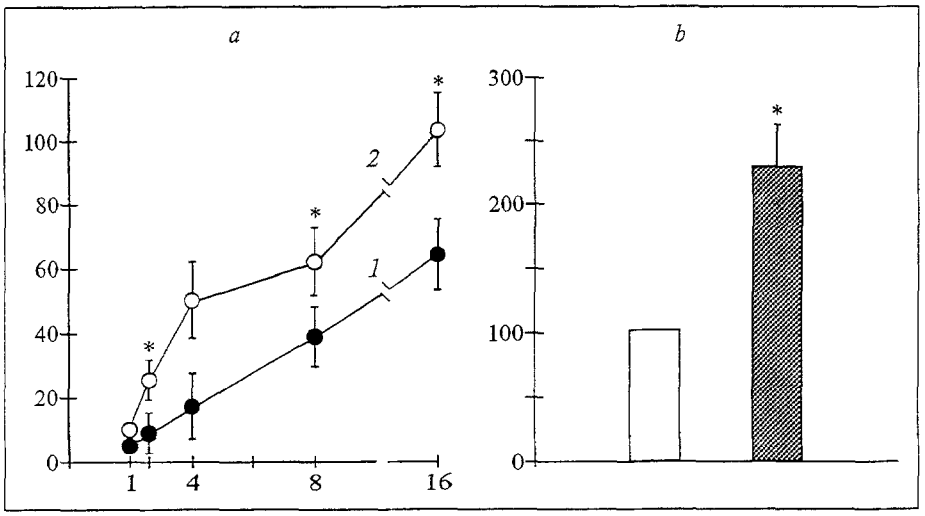

Fig. 1. Effect of ET−1 on adrenoreactivity of caudal artery. *a*) changes of perfusion pressure (P) in response to stimulation of sympathetic nerves. *1*) control (сплошная линия, solid line); *2*) against the background of ET−1 (5×10^{-10} M) (прерывистая линия, broken line). Abscissa: stimulation frequency, Hz; ordinate: increase of P, mm Hg. *b*) changes of P (%) in caudal artery in response to infusion of phenylephrine in a dose of 5×10^{-7} M. *1*) control; *2*) against the background of ET−1. Control response is taken as 100%. Here and in Tables 2−3: an asterisk denotes $p<0.05$ vs. control.

ends with pins. The lumen of the artery was perfused at a constant flow rate of 2 ml/min using a Rabbit peristaltic pump (USA). The agents studied were infused in the lumen of the vessel perfused. The changes of perfusion pressure (P) which arose for varying the lumen of the vessel were recorded with a Statham P23ID transducer on a Nihon Kohden polygraph (Japan). Use was made of aerated (4% O_2 + 96% CO_2) solution of the following composition (mM): NaCl 130, d-glucose 11, $NaHCO_3$ 14.9, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, and KH_2PO_4 1.18. Phenylephrine (PE), norepinephrine, HCl, staurosporin, and vasopressin (Sigma), sodium fluoride (Poland), endothelin (Nowa, Switzerland) were also used in the study.

RESULTS

In both the strip of the thoracic aorta and the caudal artery ET caused a slowly developing dose-dependent contraction strongly resistant to the removal of ET-1 from the solution. After 1 hour in just 80% of cases the mechanical tension in the aorta dropped to a level close to the initial one. The repeated response to ET-1 after removal of the preparation was markedly weaker than the first one and constituted just 30.7±9.1% of the latter for an average concentration of ET-1 of 5×10^{-9} M. This fact, namely, desensitization of the receptors, which is well documented in the literature [8,11], makes it impossible to study several contractile responses to ET on one preparation, using the first response as the control. Therefore, we assessed the value of the contractile response to ET as the percentage of the amplitude of a control contraction of the same preparation in the presence of 1 mM of the α-agonist PE.

In a comparison of the dynamics of the contractile responses of the aorta caused by ET, PE, or KCl, the following specificities of ET-induced contraction were discovered: first, the absence of a pronounced phasic component is typical of ET-1; second, the amplitude of contraction for the effect of maximum and half-maximum concentrations of ET-1 was markedly higher. The latter is evidence that even the smallest amounts of ET-1 produced by the vascular endothelium may markedly alter the tone of the vascular smooth muscles, which, in turn, may affect the responsiveness of the vessels to other vasoconstrictors. In fact, we observed potentiation of the contractile response while investigating the effect of ET-1 on the adrenoreactivity of the caudal artery (Fig. 1, *a*, *b*). The experimental protocol was the following. After the recording of the control responses to stimulation of the sympathetic nerve endings in the adventitia of the vessel (parameters of stimulation with an electric field (current 0.4 A, frequency 1-16 Hz, pulse duration 1 msec, total duration 10 sec) or after the recording of the test contraction in response to PE in a dose of $ED_{50}=5\times10^{-7}$, the vessel was incubated for 15 min in a solution containing ET-1, which was added to the perfusion fluid in a subthreshold concentration of 5×10^{-10} M. This concentration did not raise the initial level of the perfusion pressure in the vessel by more than 1-5 mm Hg. Against the background of continuous infusion of ET-1, the stimulation of the sympathetic nerves or the addition of PE was then repeated. These repeated responses were 1.5-2 times higher than the first ones (Fig. 1).

Evidently, the functional role of ET-1 in the endothelium-vascular smooth muscle system is not confined to the constrictive effect per se. ET-1 exerts the effect upon the responsiveness of the smooth muscle cells to a number of other agonists: it potentiates responses to adrenergic effects [6] (Fig. 1), and to serotonin, histamine, and vaso-

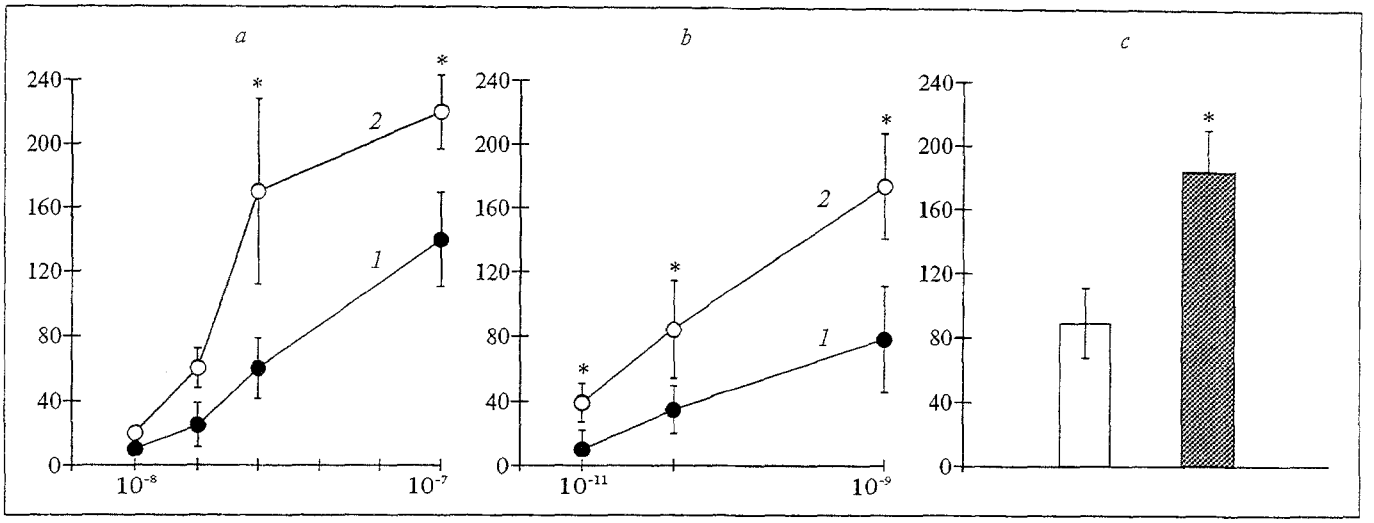

Fig. 2. Potentiation of contractile response (%) against the background of NaF (2 mM) for the effect of phenylephrine (*a*), vasopressin (*b*), and ET−1 (5×10^{-8} M) (*c*). ET−1 response was assessed in % of test response to phenylephrine. *1*) control; *2*) NaF. Abscissa: concentration of agonist, M; ordinate: increase of P, mm Hg.

pressin [15]. The enhanced vasoconstriction for PE or for stimulation of the sympathetic nerves against the background of ET-1 (Fig. 1) may be due to the fact that ET-1, even in very low, subthreshold doses, is able to initiate slow Ca^{2+} entry via the voltage-dependent channels [10]. The ability of ET-1 to increase the calcium sensitivity of contractile protein phosphorylation in the presence of guanosine triphosphate (GTP) cannot be ruled out; at the same time, the contraction of smooth muscle cells may develop in a calcium-free medium at an intracellular Ca^{2+} level close to the resting one [5].

The specificities of the physiological effect of ET-1, i.e., the slow relaxation of cells and their rapid desensitization to ET discovered in our experiments, may be due to internalization of the ET-receptor complex in the membrane, the slow metabolism of ET-1 in the vascular tissue, and depletion of intracellular transmitters of contraction [7,8]. Desensitization is evidently not associated with the functional changes of PKC, since an inhibitor of this kinase, H7, does not affect the degree of desensitization [3].

Inositol triphosphate (IP_3), a product of hydrolysis of inositol-containing phospholipids, is known to play a crucial role in the mobilization of intracellular Ca^{2+}, which is necessary for triggering contraction [10]. This hydrolysis proceeds under the influence of phospholipase C (PLC), which may be activated in the presence of Ca^{2+}-mobilizing agonists by several pathways, in particular, via the GTP-binding membrane proteins (G-proteins) [16]. In the experiments on the perfused rat caudal artery we studied the effect of NaF on the ET-induced contraction of the smooth muscles. The F ion interacts with the α-subunit of G-protein, this simulating the GTP molecule at the binding sites and thereby activating this protein [16]. NaF in a subthreshold dose of 2 mM, which did not per se affect the vascular tone, increased the responsiveness of the smooth muscle cells to PE, vasopressin, and ET-1 (Fig. 2).

Among the mechanisms of action of ET-1 an important role is played by another product of phosphoinositide hydrolysis, diacylglycerol, which is an activator of calcium-phospholipid-dependent PKC. To study the role of this kinase in the

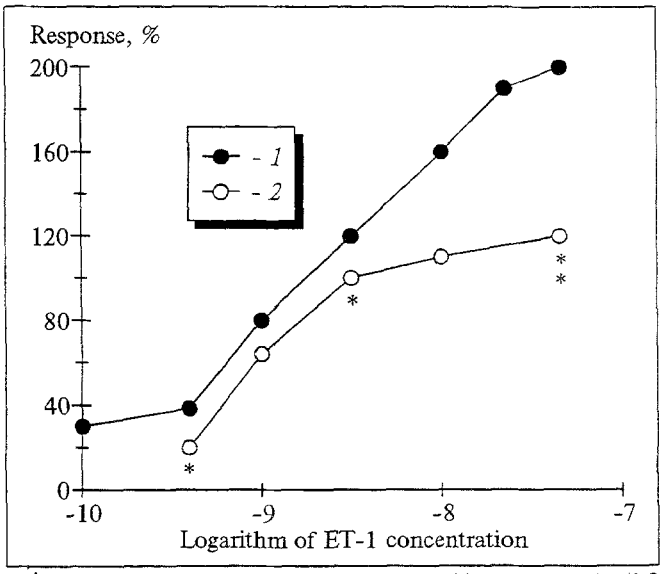

Fig. 3. Effect of incubation of aorta strip with staurosporin (0.2 mM) on ET−1−induced contraction. *1*) control; *2*) staurosporin. ordinate: response, % of amplitude of contraction induced by 1 mM phenylephrine. Two asterisks denote $p<0.01$ vs. control.

contraction of smooth muscles of the aorta, we incubated an aorta strip with 1 mM of staurosporin, a blocker of this enzyme, for 30 min. In our experiments depression of the activity of PKC markedly suppressed the ET-1-induced contraction of the preparation (Fig. 3). It should be mentioned that PE-induced contraction was not responsive to staurosporin.

On the basis of our findings and the data of other scientists, several assumptions may be made about the ET-1-mediated pathways of the intracellular signal transfer. Apparently, by interacting with the receptor, ET-1 activates the membrane G-proteins, this being confirmed by other researchers in studies on a culture of smooth muscle cells [1,14]. Probably, it is the G_p-protein, since the ET-1-induced contraction is enhanced against the background of the F ion and is scarcely inhibited by the pertussis toxin, which inactivates the G_o- and G_i-proteins [14]. Activation of PLC and IP_3 accumulation result in a release of Ca^{2+} from the intracellular sources and in Ca^{2+}-calmodulin-dependent phosphorylation of the light chains of myosin. Possibly, G-protein is not only linked with PLC, but may also directly stimulate Ca^{2+} entry via the voltage-dependent channels [1,16]. The increased production of diacylglycerol in the cell leads to the activation of calcium-phospholipid-dependent PKC, which, in accordance with our data, is a prerequisite for the development of long-term and strong contraction of cells.

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