Physiological Concentrations of Endothelin-1 Cause Only Coronary Vasodilatation in Anesthetized Rats

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Various concentrations of the peptide endothelin-1 were tested for their effect on coronary vascular resistance in anesthetized rats. Intracoronary infusion of this peptide over 4 min in a total dose that gave rise to blood concentrations of 0.002-0.003 or 0.2-0.3 fmol/ml elicited only a decrease in the estimated coronary vascular resistance. It is suggested that only supraphysiological doses of endothelin are likely to exert a powerful vasoconstrictive effect on coronary vessels and cause myocardial ischemia.

Key Words: *endothelin-1; physiological concentrations; coronary circulation; vascular resistance*

Endothelin, a peptide of 21 amino acid residues first isolated in 1988 from cultured endothelial cells of the porcine aorta [13], can alter arterial pressure (AP), cause bronchospasm, exert positive ino- and chronotropic effects, and modulate the transmission of neural signals [14]. Awake animals administered this peptide in bolus form show a biphasic change in AP, whereby its transient fall is succeeded by a sharp and persistent rise [1,10, 13]. It is believed that such a biphasic response is most likely due to the activation of two different types of endothelin receptors, and that receptors of one type (ETb) are located on the vascular endothelium and, when activated, cause endothelium-dependent vasodilatation [11], while those of the other type (ETa) occur in smooth-muscle cells and are responsible for their endothelin-induced contraction [3]. This suggests that low plasma concentrations of endogenous endothelin can only cause a decrease in vascular tone. However, endothelin circulating in the blood may be implicated in the genesis of coronary vasospasm, as is suggested by experiments where the action of endothelin was studied in animals given it in relatively high doses [4,5,8] so that its concentrations in the blood reached levels which probably never occur under physiological or pathophysiological conditions [4].

The purpose the present study was to evaluate the effects of endothelin-1 in relatively low doses on the coronary vascular bed in anesthetized rats.

MATERIALS AND METHODS

Nembutal-anesthetized random-bred male albino rats weighing 250-330 g were used. The effects of endothelin-1 were studied using our modification of the method by which coronary vessels are perfused with the animal's own blood [2]. Blood for autoperfusion was taken from the left carotid artery with a catheter connected to a shunting system (Fig. 1, *a*, *1*). A glass catheter was introduced into the aortic area via the right carotid artery and then carefully manipulated into the left coronary artery (Fig. 1, *b*). A transient fall in AP and the commencement of blood flow through the shunting system indicated that the catheter was in the right place. The intracoronary infusion of endothelin-1 or physiological saline was continued for 4 min at a rate of 0.04 ml/min using an infusing device. During the experiment, systemic AP, coronary blood flow, and perfusion pressure in the system were recorded.

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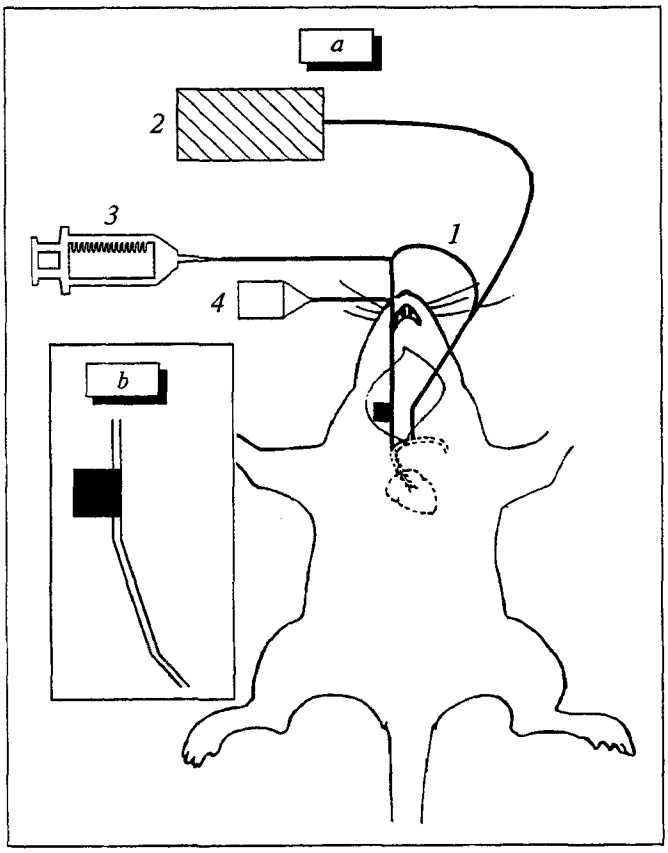

Fig. 1. Schematic of the model used for the autoperfusion of coronary vessels in anesthetized rats. *a*) general experimental setup: *1*) perfusion loop, *2*) Doppler blood flow meter, *3*) infusing device, *4*) AP sensor; *b*) enlarged representation of the glass catheter.

The experimental protocol involved infusion of physiological saline and endothelin-1 in total amounts of 0.2 fmol, 20 fmol, 2 pmol, and 0.2 nmol per kg body weight, at intervals of 30-40 min. Coronary vascular resistance (CVR) was calculated as the ratio of perfusion pressure to coronary blood flow minus resistance of the system. The results were subjected to statistical analysis using Student's *t* test for paired samples.

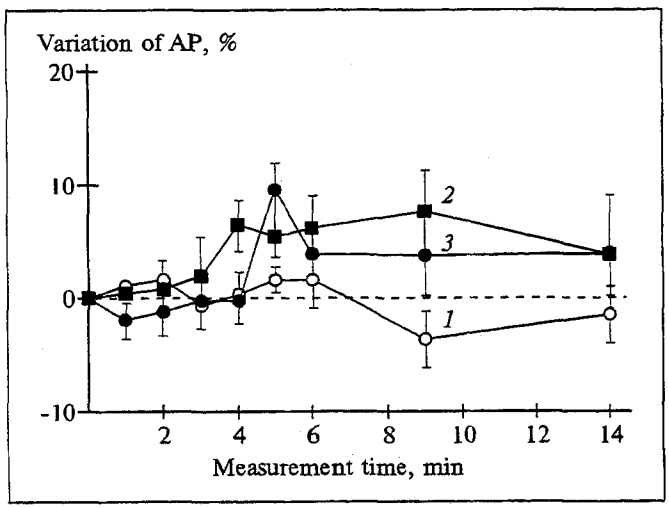

Fig. 2. Effect of intracoronary endothelin-1 infusion in different doses on AP in anesthetized rats. Here and in Fig. 3: rats were given endothelin-1 in a total dose of 0.2 fmol/kg (*1*), 20 fmol/kg (*2*), and 2 pmol/kg (*3*), and the abscissa indicates measurement times during (minutes 0-4) and after (minutes 4-14) endothelin infusion.

RESULTS

The total coronary vascular resistance measured in 10 rats had a mean value of 52.66±9.56 mm Hg (range, 16.36-70.51 mm Hg), and the mean AP in these rats was 125.9±4.91 mm Hg. Intracoronary infusion of either saline or endothelin-1 in doses of 0.2 fmol/kg to 0.2 nmol/kg did not cause significant changes in the recorded parameters. However, the pressor effect of the peptide tended to increase as more of it was infused (Fig. 2). The greatest change in AP (by 13.05%) as compared to the baseline (preinfusion) level occurred approximately at minute 5 after the discontinuation of infusion.

Endothelin-1 reduced CVR throughout the infusion period, after which CVR rose to exceed the baseline level for a short time (minutes 10-15). These biphasic changes in CVR varied in degree depending on the endothelin-1 doses used (Fig. 3). After the doses of 0.2 and 20 fmol/kg, CVR decreased by 13.6±4.9% and 20.6±6.5%, respectively ($p<0.05$, $n=6$), followed by a rise to the control level. Similar biphasic changes in CVR (an increase by 12.5±5.5% and then a decrease by 20.5±9%) were observed after the dose of 2 pmol/kg (endothelin-1 concentrations in the coronary bed then ranged from 20 to 30 fmol/ml; $n=5$), but the differences from the control tests with physiological saline were insignificant. Infusion of the peptide at 0.2 nmol/kg (its concentration in the blood flow averaged 2-3 pmol/ml; $n=2$) led to a slight reduction of CVR followed by pronounced (62%) constriction of the coronary vessels. Vascular resistance rose at minutes 3 and 4 of infusion and remained elevated for 10 min postinfusion, i.e., during the whole period when parameter values were calculated.

In this study, intracoronary endothelin-1 infusions over 4 min in total doses of 0.2 fmol/kg, 20 fmol/kg, and 2 pmol/kg altered CVR without causing significant changes in systemic AP, and it was therefore possible to study the local effect of this peptide. Because the blood flow level was measured continuously, concentrations of the exogenously administered peptide simulating those of the endogenous peptide in the blood under various conditions could be calculated.

Endothelin-1 levels in the blood plasma of healthy people have been reported to range from

0.36 to 1.50 fmol/ml. Higher levels are recorded in a variety of disease states, including cardiogenic shock, heart failure, and pulmonary edema [12], diffuse intravascular thrombus formation [7], diabetes [6], and some forms of hypertension [9]. Endothelin-1 concentrations in disease do not exceed those in health by a factor of more than 10 [7]; when they reach levels in excess of 5 fmol/ml, death occurs - at least among patients with extensive myocardial infarction [12]. On the basis of this reported evidence, we evaluated in the present study the effects of both physiological and pathophysiological endothelin-1 concentrations in the blood, namely 0.002-0.003 fmol/ml (in rats given a total dose of 0.2 fmol/kg), 0.2-0.3 fmol/ml (total dose = 20 fmol/kg), 20-30 fmol/ml (total dose = 2 pmol/kg), and 2-3 pmol/ml (total dose = 0.2 nmol/kg). The 4-minute infusion of the two lowest doses led only to coronary vasodilatation: the maximal decreases in total CVR were 13.6±4.9% and 20.6±6.5%, respectively, relative to the preinfusion levels ($p<0.05$).

Infusing the higher dose of 2 pmol/kg, which resulted in local endothelin-1 concentrations of 20-30 fmol/ml, caused an insignificant (by 12.5±5.5%) vasodilation during the infusion and was followed by a more strongly marked, though also insignificant, vasoconstriction (CVR rose by 20.6±6.5%). Presumably, the peptide circulating in such concentrations passes through the vascular endothelium in amounts sufficient to activate the specific receptors of smooth muscle and to cause vasoconstriction. This late contractile response appears to mask the rapidly occurring vasodilatation. Moreover, the only effect from the largest of the doses used (0.2 nmol/kg), which gave rise to the highest blood concentrations of endothelin-1 (2-3 pmol/ml), was pronounced coronary vasoconstriction (CVR rose by 62%), which was already observed during the last minute of infusion. The 3-minute period preceding the onset of the contractile response to such high endothelin-1 concentrations in the blood may have been the time it took the peptide to penetrate through the endothelial monolayer.

The results of this study indicate that intracoronary infusions of endothelin-1 in doses giving rise to blood concentrations similar to those occurring in health or disease produce only a vasodilatative effect. According to the literature, this effect of endothelin-1 is most likely mediated by the receptors located on the vascular endothelium (ETb-type receptors). The endothelin-1 acting on these receptors activates the synthesis and release by the endothelium of vasodilating factors (prostaglandin and nitric oxide), and these cause relaxation of smooth-muscle cells. It may be concluded that even the considerable elevations of plasma endothelin-1 levels occurring in various disease states cannot be responsible for the extensive cardiac ischemia documented in experiments using unacceptably high doses of this peptide.

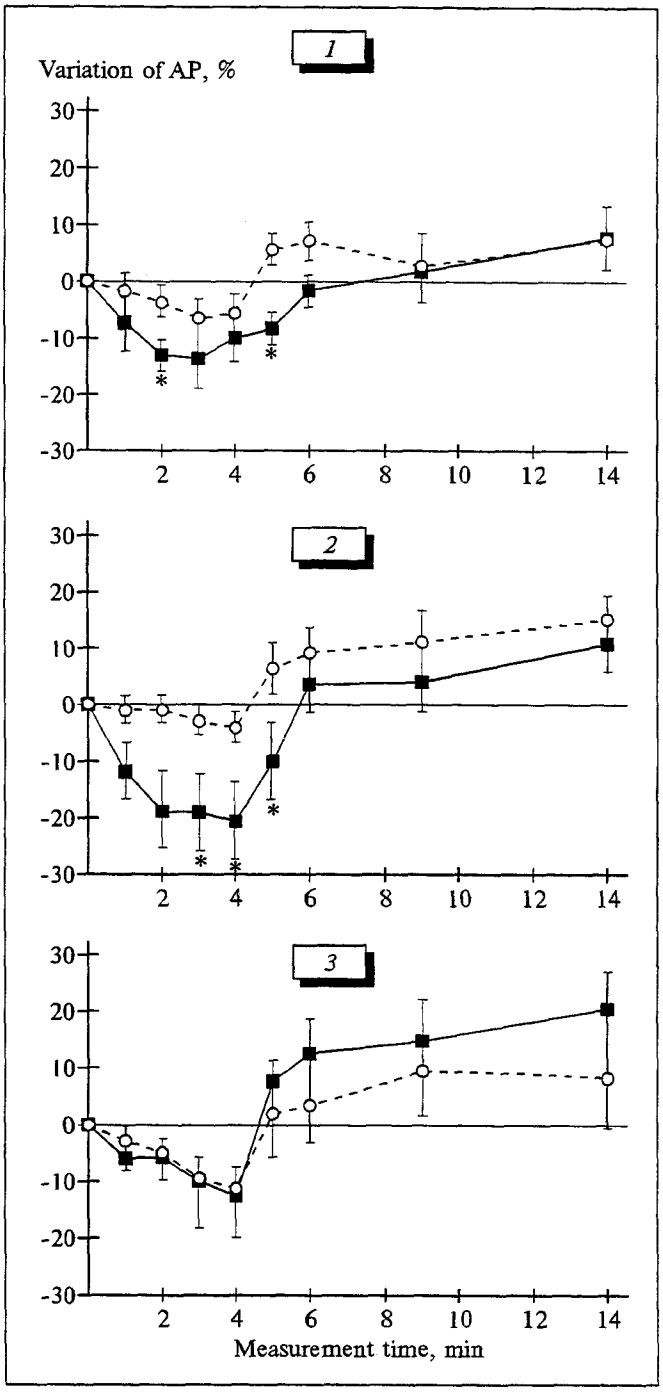

Fig. 3. Impact of 4-minute endothelin-1 infusion in different doses on coronary vascular resistance (CVR) in anesthetized rats. *Solid line*: endothelin-1 infusion; *dashed line*: infusion of physiological saline (control group). The asterisk denotes a significant difference at $p<0.05$ from the control group.

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