Effects of Dose and Administration Mode of Endothelin 1 on the Mean Arterial Pressure and Heart Rate in Awake Rats

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The effects of bolus administration and short-term infusion of endothelin 1 in four doses (2×10^{-16}, 2×10^{-14}, 2×10^{-12}, and 2×10^{-10} mol/kg) on arterial pressure and heart rate were compared in awake rats. Infusion and bolus administration of the two highest doses increased arterial pressure and provoked bradycardia. Infusion of the two lowest doses increased heart rate without concomitant changes in arterial pressure, while bolus injection of endothelin 1 in the same doses decreased both arterial pressure and the heart rate.

Key Words: *endothelin, hemodynamics*

Endothelin (ET) is an endogenous peptide consisting of 21 amino acid residues. It was originally isolated in 1988 from cultured endothelial cells of porcine aorta and has been intensely studied as a powerful vasoactive agent [9]. Three forms of this peptide were identified (ET-1, ET-2, and ET-3) and three genes were cloned which are responsible for their synthesis [3,9]. ET-1 is the basic form of this peptide that circulates in human, bovine, and other animals' blood and is presumably produced by endothelial cells. The ET content in different organs of rats varies from 0.39 to 8.7 pg/mg [1]. Depending on species and method, 10^{-15} to 10^{-13} mol ET/ml is detected in blood plasma [6]. At present, three types of ET-sensitive receptors were found [2,5,8].

In the *in vivo* experiments, ET in concentrations of 10^{-10} and 10^{-8} mol/kg increased both arterial pressure (AP) and overall resistance of peripheral vessels [9]. It also produced positive ino- and chronotropic effects on cultured cardiomyocytes and significantly increased cardiac output and heart rate (HR) without affecting the stroke volume in isolated heart [7]. The cardiac and vascular responses to high doses of ET were very long. In addition to vasoconstrictor affect, ET caused vasodilation [4] which is believed to be characteristic of low concentrations of the peptide, i.e., of the order of 10^{-15} and 10^{-13} mol/kg. There are data on ambiguity of the cardiovascular response to exogenous ET. In a number of studies it can be related to high pharmacological concentrations of the peptide, which are much higher than its blood level in humans and animals, both under normal and pathological conditions. Various modes of administration (bolus and stream injection or infusion) result in different dilution of exogenous peptide in blood, and this difference explains the specific individual vascular response. The aim of the present work was to study the responses of AP and HR to bolus injection and short-term infusion of ET-1 in doses comparable to physiological blood level of ET.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 180-200 g. To record systemic AP and HR and to administer the peptide, polyethylene catheters (PE-50, internal diameter 0.58 mm) were implanted under Nembutal anesthesia (40 mg/kg intraperitoneally) into the carotid artery and jugular vein, re-

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spectively, one day before experiment. During experiment the animals were wakeful. AP and HR were measured continuously with a Statham pressure transducer and recordered in an H3030-1 recorder. A VEDA-2 infusor was used for administration of the peptide.

Four series of experiments were carried out with the following doses of ET-1 (in mol/kg): 2×10^{-16} (n=5), 2×10^{-14} (n=6), 2×10^{-12} (n=6), and 2×10^{-10} (n=7).

Protocol of experiment:

Beginning of experiment

30 min

Infusion of 0.9% NaCl during 6 min →←

30 min

→←

Infusion of peptide during 6 min

60 min

Bolus injection of 0.9% NaCl →

30 min

Bolus injection of peptide →

60 min

End of experiment

Total duration 3.5 hours

Infusion was performed at a rate of 43 μl/min; volume of the injectate was 258 μl in each administration.

RESULTS

In all series, in the absence of any additional physiological stimuli the mean values of AP and HR were 120±4.8 mm Hg and 384.4±17.5 beats/min, respectively (n=24). Both infusion and bolus injection of physiological solution did not cause significant changes in the mean systemic AP and HR.

Infusion of ET-1 in doses of 2×10^{-10} and 2×10^{-12} mol/kg led to a significant increase in the mean AP. The maximum pressor response was 10.1± 3.8% against the background level on the 7th min from the beginning of infusion, and 4.3±1.7% on the 5th min, respectively (Fig. 1, *a*). During this period, HR declined drastically by 9.8±2.5% for ET-1 in a dose of 2×10^{-10} mol/kg, and by 4.5±0.7% for ET-1 in a dose 2×10^{-12} mol/kg (Fig. 1, *c*). Infusion of ET-1 in doses of 2×10^{-14} and 2×10^{-16} mol/kg resulted in a slight elevation against the background level of the mean AP (Fig. 1, *b*) on the 6th min by 2.3±1.7 and 1.3±1.3%, respectively, accompanied by an increase in HR (Fig. 1, *d*), which on the 6th min was 6.0±2.6% for 2×10^{-14} mol/kg, and 5.7±2.3% for 2×10^{-16} mol/kg.

Bolus injection of ET-1 caused qualitatively different dynamic changes in the mean systemic AP and HR (Fig. 2). It induced a two-phase response of the mean AP, in which an early transitory decrease in AP was followed by a pressure elevation, the value of which depended on the dose of injected peptide. The duration of the first phase was 30 sec with maximum of depressor response at 15 sec. The maximum decrease in AP was 9.2±2.0 and 2.1±0.7% for doses 2×10^{-10} and 2×10^{-12} mol/kg, and 2.5±0.8 and 3.9±1.7% for doses 2×10^{-14} and 2×10^{-16} mol/kg, respectively. Bolus injection of 2×10^{-10} or 2×10^{-12} mol/kg induced pressure elevation followed by a prolonged increase in AP with maxima minutes 1 and 2, respectively. AP increased, respectively, by 9.9±3.8 and 3.9±1.1% (Fig. 2, *a*). After 2×10^{-10} mol/kg ET-1, HR decreased with a maximum response of 7.7±1.4% (n=7) on the 6th min. Bolus injection of ET-1 in a dose of 2×10^{-12} mol/kg did not produce significant changes in HR (Fig. 2, *c*). The change in the mean AP in response to bolus injection of ET-1 in doses of 2×10^{-14} or 2×10^{-16} mol/kg was somewhat different. After an early transitory decrease, AP returned practically to the initial level, and then again demonstrated a tendency to a decrease. Decline of AP (against the background level) was 3.0±0.8% (n=6) for 2×10^{-14} mol/kg, which differed significantly from the control value on minutes 5 and 6. For the dose of 2×10^{-16} mol/kg, AP decreased by 5.0± 1.6% on minutes 6 and 7 (Fig. 2, *b*). HR also declined, and the maximum significant decrease against the background level was 4.3±1.6% (n=6) and 5.2± 2.7% (n=5) for ET-1 in doses of 2×10^{-14} or 2×10^{-16} mol/kg (Fig. 2, *d*).

Thus, both bolus injection of ET-1, and short-term infusion of this peptide in high doses (2×10^{-10} and 2×10^{-12} mol/kg) finally caused a significant increase in the mean systemic AP and a simultaneous decrease in HR due to presumably baroreceptor reflex evoked by a large enhancement of the mean AP, which attenuated and stabilized AP.

The results obtained with infusion and bolus injection of ET-1 in doses of 2×10^{-14} or 2×10^{-16} mol/kg were not so unambiguous, and they differed qualitatively from that described above. Infusion of ET-1 in these doses caused just a very small increase in AP, while HR increased significantly. Direct positive cardiac ino- and chronotropic effects of ET-1 have been discussed in the literature, so it can be

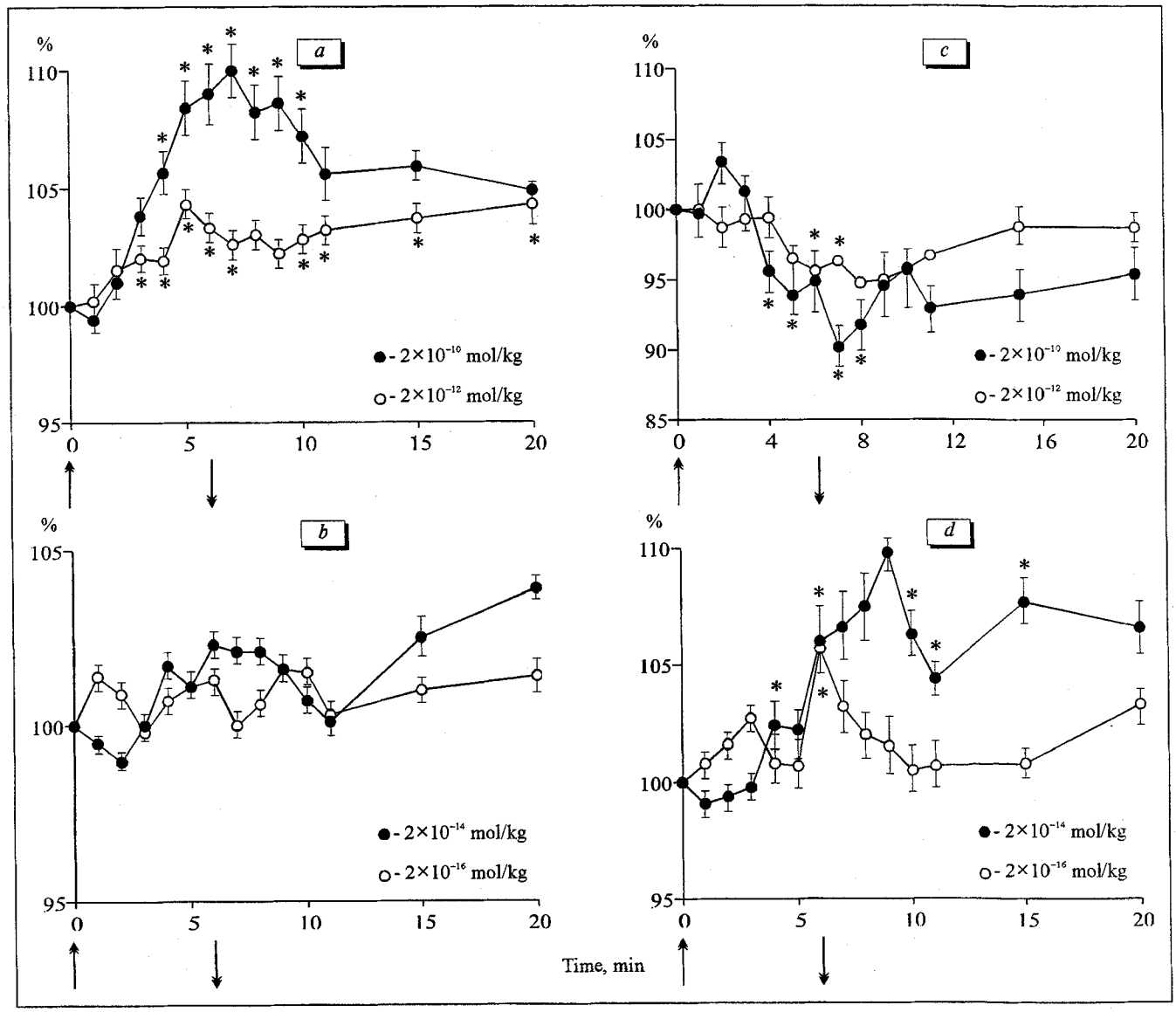

Fig. 1. Changes in arterial pressure (*a*, *b*) and heart rate (*c*, *d*) in awake rats caused by infusion of endothelin 1 in doses of 2×10^{-10} and 2×10^{-12} mol/kg (*a*, *c*), as well as 2×10^{-14} and 2×10^{-16} mol/kg (*b*, *d*). The upward arrow indicates the beginning of peptide administration, the downward arrow indicates the end of infusion. Here and in Fig. 2: *$p<0.05$ compared with the control.

suggested that in the absence of marked changes in the mean AP, increment in HR can be produced by a direct action of ET-1 on the myocardium. Bolus injection of ET-1 at low doses of 2×10^{-14} or 2×10^{-16} mol/kg led to a decline in both mean AP and HR. Under our experimental conditions, bolus injection and infusion differed only in the rate of administration, while the overall dose of the administered peptide was the same. It seems possible that by varying the rate of peptide administration, we changed its effective concentration corresponding to different types of ET-1 receptors, which could result in different degrees of their activation, and produce various responses to bolus injection and infusion of ET-1 at low doses in the awake rats. Thus, this study showed that the responses of the mean systemic AP and HR in awake rats to ET-1 depend on dose and mode of administration of the peptide.

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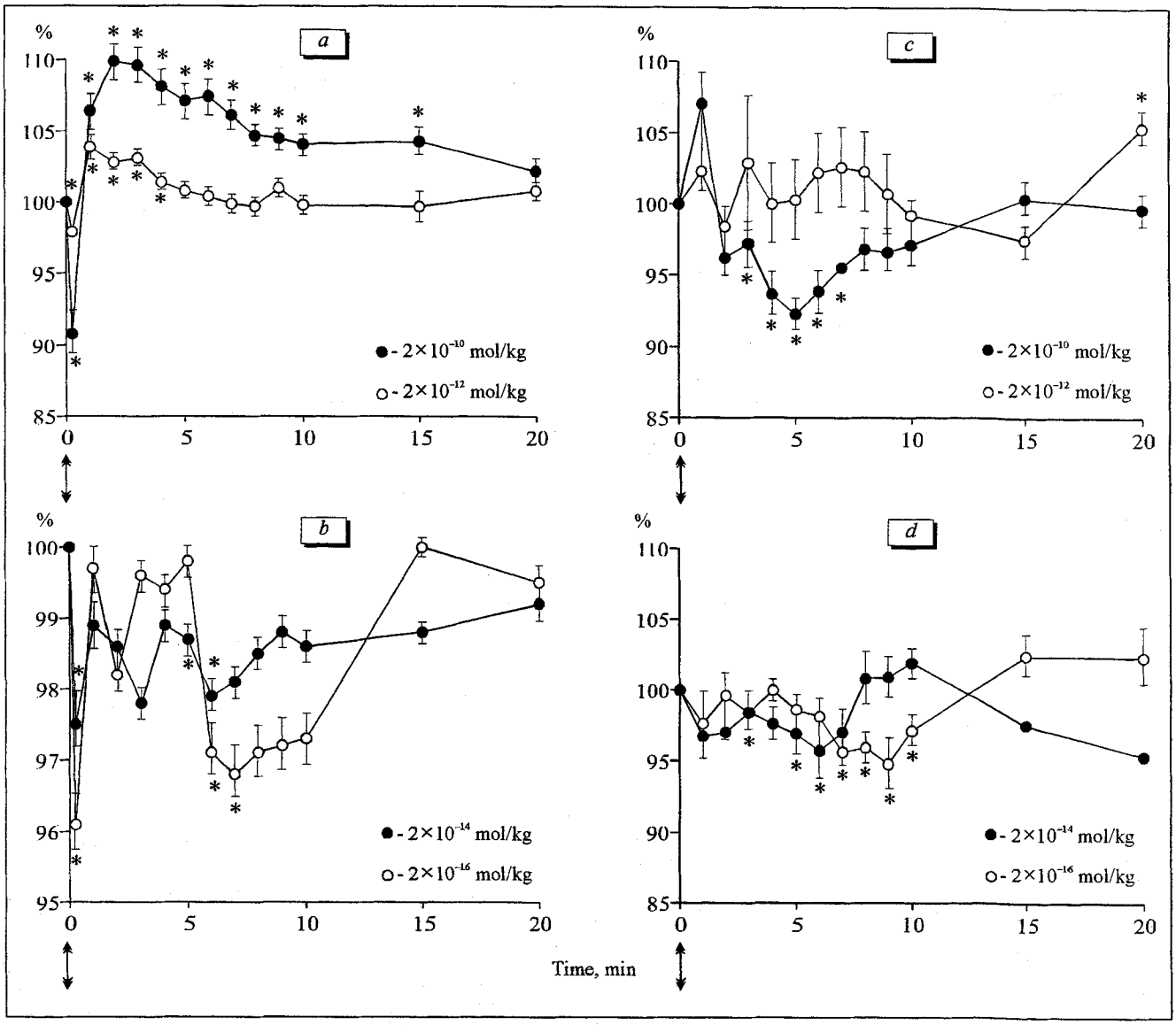

Fig. 2. Changes in arterial pressure (*a*, *b*) and heart rate (*c*, *d*) in awake rats caused by bolus administration of endothelin 1 in doses of 2×10^{-10} and 2×10^{-12} mol/kg (*a*, *c*), as well as 2×10^{-14} and 2×10^{-16} mol/kg (*b*, *d*). The arrow indicates the moment of peptide administration.

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