Arrhythmogenic Effects of Endothelin-1 under Conditions of NO-Synthase Blockade with L-NAME in NMRI Mice

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Arrythmogenic effects of endothelin-1 were studied in NMRI mice under conditions of NO-synthase blockade with Nω-nitro-L-arginine methyl ester. Intravenous injection of endothelin-1 increased heart rate variability in awake mice. NO-synthase blockade potentiated the arrythmogenic effects of endothelin-1. In narcotized animals the arrythmogenic effect of endothelin-1 was not observed and was considerably weakened under conditions of NO-synthase blockade. Arrhythmia was paralleled by atrioventricular block and lengthening of the ST segment.

Key Words: *endothelin-1; nitrogen oxide; L-NAME; mice; arrhythmia*

Endothelin-1 (ET-1) produced by endothelial cells induces cardiac arrhythmia by directly affecting the cardiac conduction system and through coronary ischemia. The direct effect of ET-1 on conducting cardiomyocytes was demonstrated using microelectrode technique of recording of transmembrane potential. Lengthening of action potentials and premature depolarization in these cells can be regarded as possible pathogenetic factors of arrhythmia caused by ET-1 [14]. Similar conclusions were made from experiments on narcotized dogs [9].

It was demonstrated that ET_A receptor blockade with BQ123 decreased the severity of arrhythmia in myocardial ischemia [4]. Nonselective blockade of ET receptors with TAK-044 also reduced reperfusion arrhythmias [5]. On the other hand, it was found that coronary ischemia and intracoronary infusion of ET-1 produced different effects on electrophysiological processes in the heart [1]. Moreover, cardiac arrhythmia preceded the development of biochemical signs of ischemia caused by intracoronary infusion of ET-1 [11]. Intracoronary infusion of ET-1 to narcotized dogs did not induce ischemic changes in ECG, but *QT* interval was prolonged [13]. Experiments on narcotized rats showed that intracoronary infusion of ET-1 caused coronary ischemia, which was seen from characteristic *ST* changes. Other disorders were atrioventricular block and extrasystoles; the experiment eventuated in ventricular fibrillation [7].

Ventricular fibrillation induced by ET-1 in isolated rat hearts can be prevented by NO donor. It is also known that NO-synthase inhibitors can modulate the effect of ET-1 on the heart by affecting cardiac contractility and coronary vessels [2]. Moreover, the blockade of NO synthesis can modulate the sympathetic and parasympathetic effects on the heart and affect the cardiac conduction system [12]. However, *in vivo* influence of endogenous NO on arrhythmogenic effects of ET-1 were not studied.

We investigated the arrhythmogenic effects of ET-1 under conditions of NO-synthase (NOS) blockade with L-NAME (Nω-nitro-L-arginine) in awake and narcotized NMRI mice.

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MATERIALS AND METHODS

Experiments were carried out on male NMRI mice weighing 30-40 g (Breeding Center of Affiliated Division of Institute of Bioorganic Chemistry) in accordance with the program on humane handling of laboratory animals developed at the Institute. Polyethylene catheters for blood pressure (BP) recording and infusions were implanted into the thoracic aorta through the left carotid artery and into the left jugular vein, respectively, under droperidol-calypsol anesthesia (3 and 63 mg/kg, respectively, intramuscularly). In some animals electrodes for ECG recording in the thoracic lead were implanted together with the catheters. Experiments were carried out on the next day after surgery on awake mice. A special series of acute experiments was carried out on narcotized animals (urethane, 1.5 g/kg intraperitoneally). Catheters were implanted to all animals in this series. ECG in the thoracic lead was recorded using needle electrodes.

The initial BP and heart rate were recorded for 15 min. Then controls were injected with 0.9% NaCl (100 μl/kg) and after 10 min the injection was repeated. Group 1 mice (n=12) were intravenously injected with 0.9% NaCl (100 μl/kg) and after 10 min with ET-1 (1 nmol/kg). The parameters were recorded for 15 min after ET-1 injection. The mice of groups 2 and 3 received intravenous injection of L-NAME (2.5 mg/kg) and after 10 min 0.9% NaCl (100 μl/kg, n=6) or ET-1 (n=12), respectively. The parameters were measured for 15 min after ET-1 injection.

BP and ECG were recorded with an electric manometer and electrocardiograph. BP and ECG curves were processed using original software. The mean BP, heart rate, wave amplitudes and intervals were estimated. Heart rate variability was determined as a standard deviation for the first 1000 cardiac cycles after drug injection. The significance of differences was evaluated using Student's t test.

RESULTS

ET-1 increased variability of cardiac rhythm in awake mice to 51±5 bpm in comparison with the control (32±2 bpm, p<0.05). NO-synthase blockade with L-NAME potentiated the arrhythmogenic effect of ET-1 (Fig. 1, *a*), under these conditions heart rhythm variability was 118±20 bpm. Normal heart rhythm in ET-treated animals was replaced with tachicardia followed by bradycardia. This was paralleled by chaotic changes in the intervals between heart contractions: they decreased forming extrasystoles or increased (Fig. 2, *b*, *c*). The prolongation of the interval between contractions can be caused by impairment of the electric pulse conduction. This phenomenon can be detected by ECG, but in awake mice ECG depends on many factors (Fig. 2, *a*, *c*), which did not allow us to unambiguously interpret the ECG data, and we therefore carried out a series of experiments on narcotized animals.

In narcotized animals the arrhythmogenic effect of ET-1 manifested only under conditions of NO-synthase blockade (Fig. 1, *b*) and was less pronounced compared to awake animals (Fig. 1, *a*, *b*). Heart rate variability in this group was 58±15 bpm. In a narcotized mouse treated with ET-1 under conditions of NO-synthase blockade and demonstrating high variability of cardiac rhythm, ECG revealed arrhythmia associated with atrioventricular blockade: *P* wave was not followed by *QRS* complex (Fig. 3). Such defective *P* waves appeared with a 55 msec delay and their amplitude was below the control (0.08±0.01 *vs.* 0.11± 0.01 mV, respectively, p<0.05). This defective *P* wave was followed (after 81±1 msec) by another *P* wave with an amplitude of 0.18±0.02 mV (higher than in the

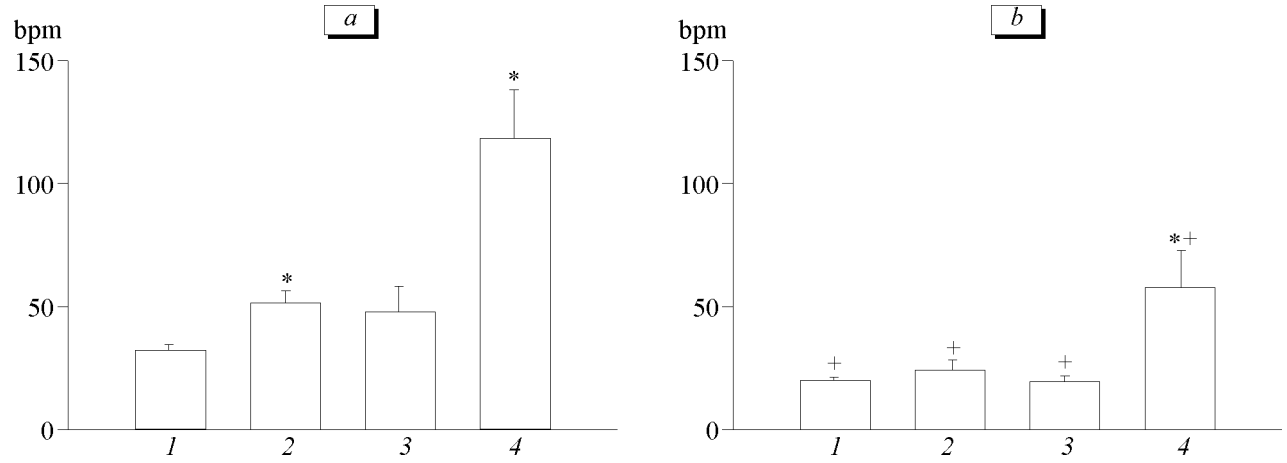

Fig. 1. Effects of endothelin-1 (ET-1, 1 nmol/kg intravenously) and L-NAME (2.5 mg/kg intravenously) on heart rate variability estimated as a standard deviation for 1000 heart contractions in awake (*a*) and urethane-narcotized (1.5 g/kg intraperitoneally) mice (*b*). *1*) control; *2*) experimental group 1; *3*) group 2; *4*) group 3. p<0.05 compared to: *animals treated with 0.9% NaCl; +awake animals.

control, $p<0.05$) and *QRS* complex, and electrical activity eventuated in a cardiac contraction. These ECG changes were observed in solitary rhythm disturbances and during bradycardia development (Fig. 3, *a*, *c*). Comparative analysis of intervals between ECG waves showed that only *ST* segment increased significantly (to 20±2 msec) in animals treated with ET-1 after L-NAME in comparison with controls (12±3 msec) and animals treated with ET-1 alone (12±4 msec).

Hence, arrhythmogenic effects of exogenous ET-1 were studied for the first time on mice. These effects manifested only in awake mice, while narcosis inhibited the arrhythmogenic effects of the peptide. Blockade of endogenous NO synthesis 2-fold potentiated the arrhythmogenic effects of ET-1 in awake mice and provoked their manifestation in narcotized animals. The effects of the NO system on arrhythmogenic effects of ET-1 can be due to its effect on coronary vessels and on the conduction system. Experiments on isolated rat hearts showed that NO donor prevented ventricular fibrillation induced by ET-1, while NOS inhibitors increased cardiotoxicity of the peptide [2]. Experiments on isolated cells of rabbit sinoatrial node showed that NO-synthase inhibitor attenuated the effects of carbamethylcholine on calcium current, while NO donor molsidomine inhibited calcium current activated by β-adrenergic stimulation [6]. In dogs blockade of NO-synthase with L-NAME significantly increased heart rate variability [10]. In mice injected with L-NAME this parameter only tended to increase. Contrary to the data that NO-synthase blockade increased heart rate variability, in neuronal NO-synthase knockout mice heart rate variability was lower than in controls [8]. The discrepancy between these results can be due to the fact that L-NAME inhibits both neuronal and endothelial NO-synthase. It is known that blockade of endothelial NO-synthase can lead to coronary ischemia, which, in turn, can cause arrhythmia.

The appearance of arrhythmogenic effects of ET-1 in narcotized mice under conditions of NO-synthase blockade in our experiments was due to the effect of the peptide on coronary vessels (which was confirmed by lengthening of *ST* segment) and on the conduction system cells (which was seen from delayed appearance of *P* wave and its low amplitude). Changes in the

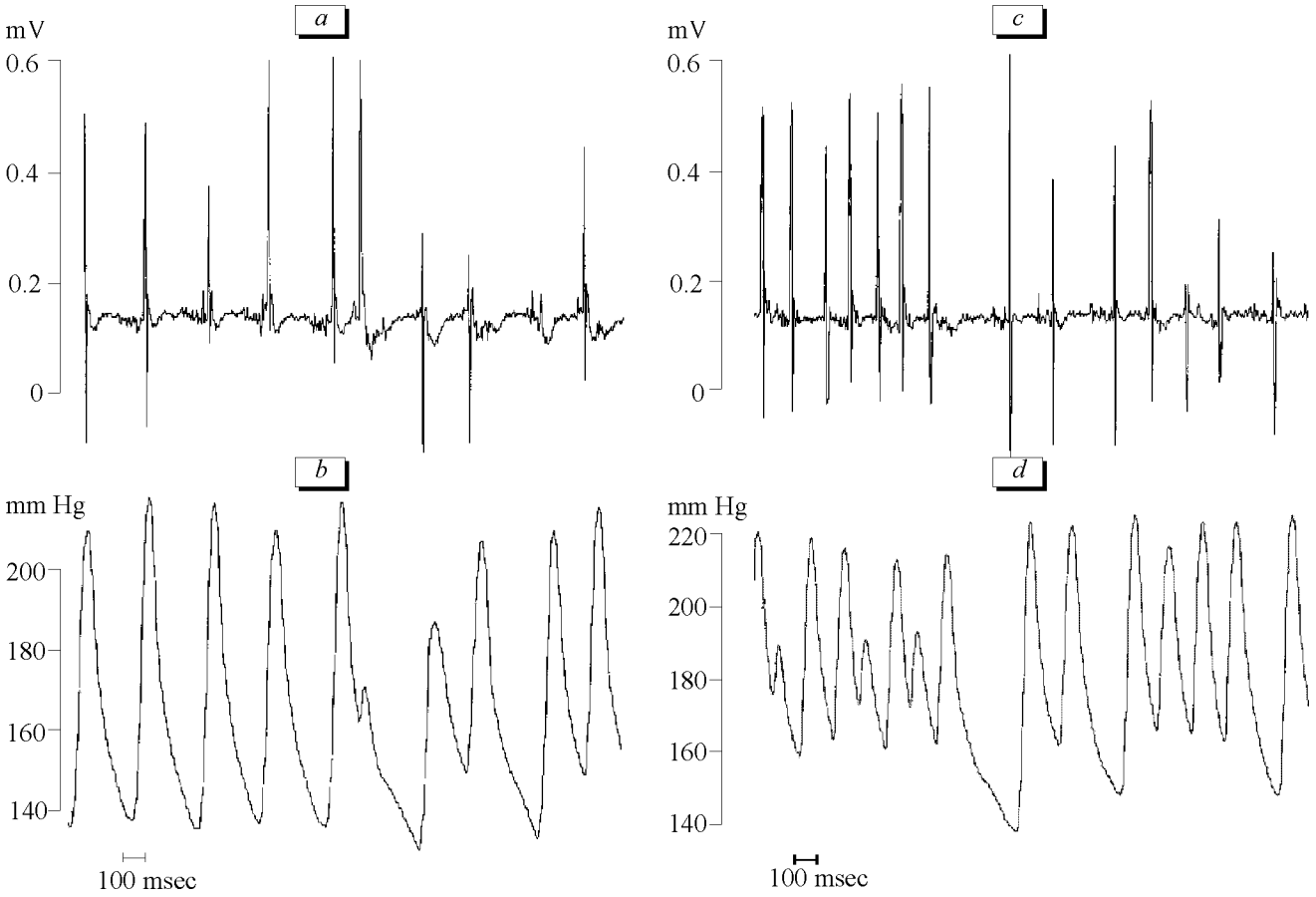

Fig. 2. ECG and arterial pressure (BP) in awake NMRI mice injected with ET-1 (1 nmol/kg intravenously) after L-NAME (2.5 mg/kg intravenously). *a*) ECG 39 sec after injection of ET-1; *b*) BP 39 sec after injection of ET-1; *c*) ECG 5 min after injection of ET-1; *d*) BP 5 min after injection of ET-1.

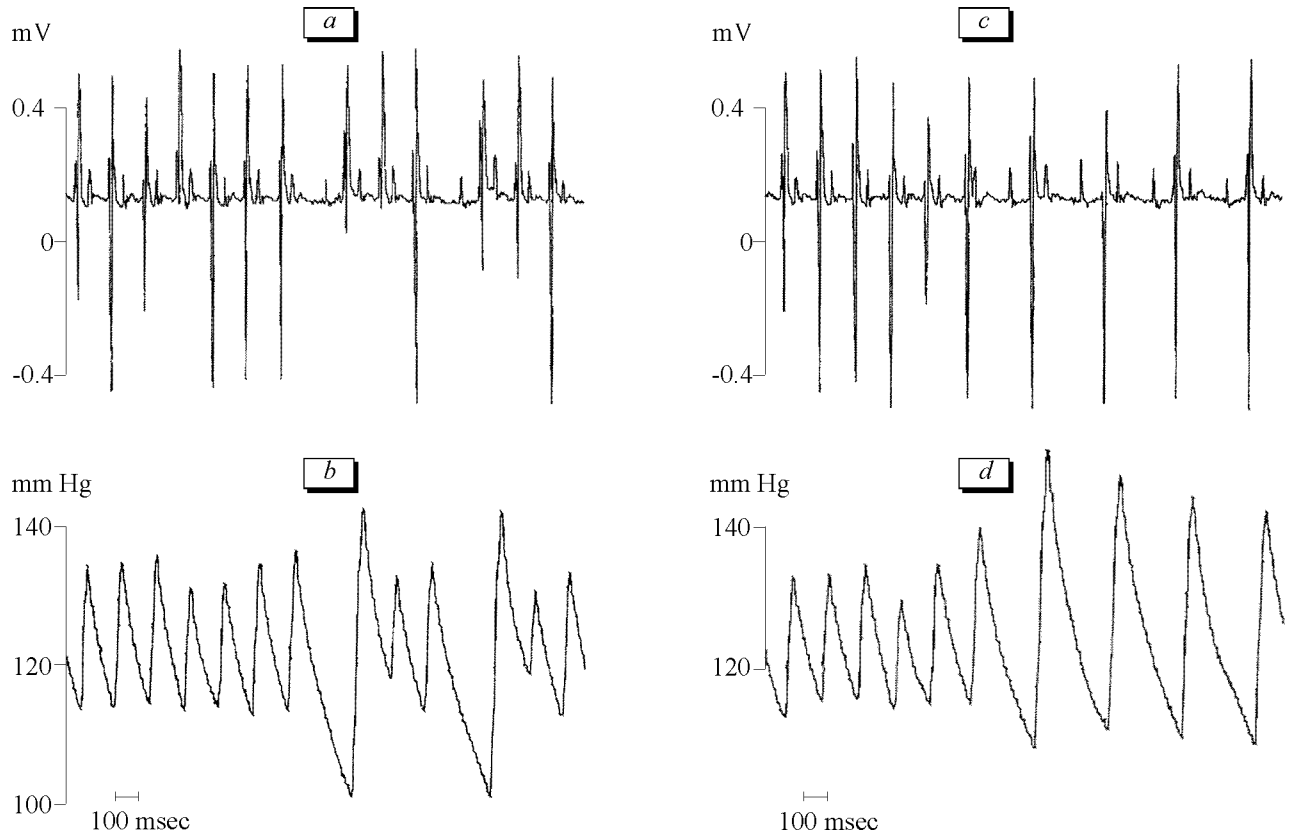

Fig. 3. ECG and BP in NMRI mice narcotized with urethane (1.5 g/kg intraperitoneally) after injection of ET-1 (1 nmol/kg intravenously) after L-NAME injection (2.5 mg/kg intravenously). *a*) ECG 18 sec after injection of ET-1; *b*) BP 18 sec after injection of ET-1; *c*) ECG 54 sec after injection of ET-1; *d*) BP 54 sec after injection of ET-1.

functional state of the cardiac conduction system are confirmed by atrioventricular block observed in mice in our study and in experiments on rats [7].

Hence, ET-1 can cause cardiac arrhythmia. This effect is more pronounced under conditions of NO-synthase blockade with L-NAME. Arrhythmia is caused by impairment of electric pulse conduction in the atrioventricular node and by coronary ischemia. The arrhythmogenic effect of ET-1 was not observed under conditions of narcosis, and its manifestation is reduced after L-NAME treatment. Presumably, the neurogenic mechanisms play an important role in the realization of the modulating effect of NOS system on ET-1-induced arrhythmias.

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