Effect of Cholinergic and Adrenergic Receptor Blockade on Arrhythmogenic Activity of Endothelin-1 during Inhibition of Nitric Oxide Synthesis in Awake Mice

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We studied the effects of blockade of nicotinic receptors in sympathetic and parasympathetic ganglia (hexamethonium), muscarinic receptors (atropine), and β_1-adrenoceptors (atenolol) on arrhythmogenic activity of endothelin-1 during inhibition of nitric oxide synthesis with Nω-nitro-L-arginine in NMRI mice. Atropine reduced, while hexamethonium completely abolished the arrhythmogenic effect of endothelin-1 during nitric oxide synthase inhibition. Atenolol potentiated arrhythmogenic activity of Nω-nitro-L-arginine, but endothelin-1 had no effect on the incidence of arrhythmias under these conditions.

Key Words: *endothelin-1; Nω-nitro-L-arginine; hexamethonium; atropine; atenolol*

Endothelin-1 (ET-1) is synthesized in cardiomyocytes, endothelial cells, and cardiac fibroblasts and plays an important role in the regulation of cardiac function [5]. ET-1 is involved in the pathogenesis of various heart diseases (*e.g.*, cardiac arrhythmias). Arrhythmogenic activity of ET-1 is associated with ischemia of coronary vessels and direct effect on the cardiac conduction system [1,6,9]. The cardiac endothelin system is closely related to the nitric oxide (NO) synthase system. NO synthase inhibitors can modulate the influence of ET-1 on the heart [2,3]. NO donors produce a protective antiarrhythmic effect on the myocardium [2,7]. Our previous experiments on NMRI mice showed that the severity of cardiac arrhythmias induced by ET-1 markedly increases during NO synthase blockade with Nω-nitro-L-arginine (L-NAME). The arrhythmogenic effect of ET-1 was completely abolished, and the severity of arrhythmias induced by ET-1 after pretreatment with L-NAME decreased in narcotized animals. These data indicate that the development of arrhythmias induced by exogenous ET-1 is mediated by neurogenic mechanisms. Blockade of NO synthesis can modulate sympathetic and parasympathetic influences on the heart [10].

Here we studied the effects of blockade of nicotinic receptors in sympathetic and parasympathetic ganglia (hexamethonium), muscarinic receptors (atropine), and β_1-adrenoceptors (atenolol) on arrhythmogenic activity of endothelin-1 during inhibition of nitric oxide synthesis in awake NMRI mice with L-NAME.

MATERIALS AND METHODS

Experiments were performed on male NMRI mice weighing 30-40 g. The study was conducted according to the requirements of the Committee for Maintenance and Use of Laboratory Animals.

The mice were intramuscularly narcotized with droperidol and calipsol in doses of 3 an 63 mg/kg, respectively. Polyethylene catheters were introduced into the thoracic aorta. Blood pressure (BP) was measured through catheters inserted through the left carotid artery. The test substances were administered via catheters implanted into the left jugular vein. Experiments were performed on awake mice on the next day

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after surgery. Baseline indexes were continuously recorded over 15 min. The measurements continued during administration of substances and for 15 min after the last injection. The first and second injections were made at a 5-min interval. The interval between the second and third injections was 10 min.

Each series was performed on 3 groups of animals (Table 1).

BP was measured using an electromanometer. Analog signals (512 Hz) were digitized on a 16-bit analog-digital converter. BP curves were processed with original software. Mean BP and heart rate (HR) were calculated. HR variability was determined as the standard deviation for the first 1000 cardiac cycles after the third injection of substances.

The data are presented as means and standard errors. Intragroup differences were evaluated by Wilcoxon *W* test. Mann—Whitney *U* test and Duncan's test were used to detect intergroup differences (ANOVA). The differences were significant at $p<0.05$.

RESULTS

HR variability in control animals of groups 1 (NaCl) and 2 (L-NAME) was 32±2 and 48±5 bpm, respectively. The increase in HR variability in group 2 mice was statistically insignificant. In group 3 animals administration of ET-1 during NO synthase blockade increased HR variability to 118±20 bpm (Fig. 1).

Blockade of nicotinic cholinoceptors in sympathetic and parasympathetic ganglia with hexamethonium decreased HR variability and completely abolished the development of arrhythmias induced by ET-1 during NO synthase blockade (Fig. 1). Blockade of muscarinic cholinoceptors with atropine also decreased HR variability. Arrhythmogenic properties of ET-1 under conditions of combined blockade of muscarinic receptors and NO synthesis were less pronounced than in animals not receiving atropine (36±6 and 118±20 bpm, respectively). However, the increase in HR variability in percents from the initial level after administration of ET-1 was 95.9% against the background of combined blockade of muscarinic receptors and NO synthesis and 153.8% after NO synthase blockade alone. Differences in these indexes were statistically insignificant (Fig. 1). HR variability decreased during β_1-adrenoceptor blockade with atenolol, but sharply increased after administration of L-NAME (60±12 bpm, 177.8%, Fig. 1). Blockade of β_1-adrenoceptors and NO synthase completely abolished the arrhythmogenic effect of ET-1.

The increase in cholinergic influences on the heart impairs conduction in the atrioventricular bundle, lengthens the effective refractory period, and causes atrioventricular block. Experiments on dogs showed that blockade of neuronal NO synthase increased vagal activity [8]. Our experiments showed that HR variability tended to increase in mice with NO synthase blockade. It was probably related to the activating effect of L-NAME on the vagus nerve. Blockade of nicotinic receptors in sympathetic and parasympathetic ganglia with hexamethonium decreased HR variability and completely abolished the arrhythmogenic effect of ET-1 observed during NO synthase blockade. In dogs hexamethonium also abolished the increase in HR variability induced by NO synthase blockade with L-NAME [8]. Spectral analysis of HR in awake mice showed that atropine decreases HR variability, while

TABLE 1. Experimental Groups

Series	Group	Treatment with preparations		
		1	2	3
Control	NaCl (*n*=12)	0.9% NaCl (100 µl/kg)	0.9% NaCl (100 µl/kg)	0.9% NaCl (100 µl/kg)
	L-NAME (*n*=12)	0.9% NaCl (100 µl/kg)	L-NAME (2.5 mg/kg)	0.9% NaCl (100 µl/kg)
	L-NAME+ET-1 (*n*=12)	0.9% NaCl (100 µl/kg)	L-NAME (2.5 mg/kg)	ET-1 (1 nmol/kg)
Hexamethonium*	NaCl (*n*=12)	Hexamethonium (20 mg/kg)	0.9% NaCl (100 µl/kg)	0.9% NaCl (100 µl/kg)
	L-NAME (*n*=8)	Hexamethonium (20 mg/kg)	L-NAME (2.5 mg/kg)	0.9% NaCl (100 µl/kg)
	L-NAME+ET-1 (*n*=8)	Hexamethonium (20 mg/kg)	L-NAME (2.5 mg/kg)	ET-1 (1 nmol/kg)
Atropine*	NaCl (*n*=10)	Atropine (1 mg/kg)	0.9% NaCl (100 µl/kg)	0.9 NaCl (100 µl/kg)
	L-NAME (*n*=8)	Atropine (1 mg/kg)	L-NAME (2.5 mg/kg)	0.9% NaCl (100 µl/kg)
	L-NAME+ET-1 (*n*=8)	Atropine (1 mg/kg)	L-NAME (2.5 mg/kg)	ET-1 (1 nmol/kg)
Atenolol*	NaCl (*n*=10)	Atenolol (2 mg/kg)	0.9% NaCl (100 µl/kg)	0.9% NaCl (100 µl/kg)
	L-NAME (*n*=8)	Atenolol (2 mg/kg)	L-NAME (2.5 mg/kg)	0.9% NaCl (100 µl/kg)
	L-NAME+ET-1 (*n*=8)	Atenolol (2 mg/kg)	L-NAME (2.5 mg/kg)	ET-1 (1 nmol/kg)

Note. *Hexamethonium, atropine, and atenolol were administered 5 min before the first substance.

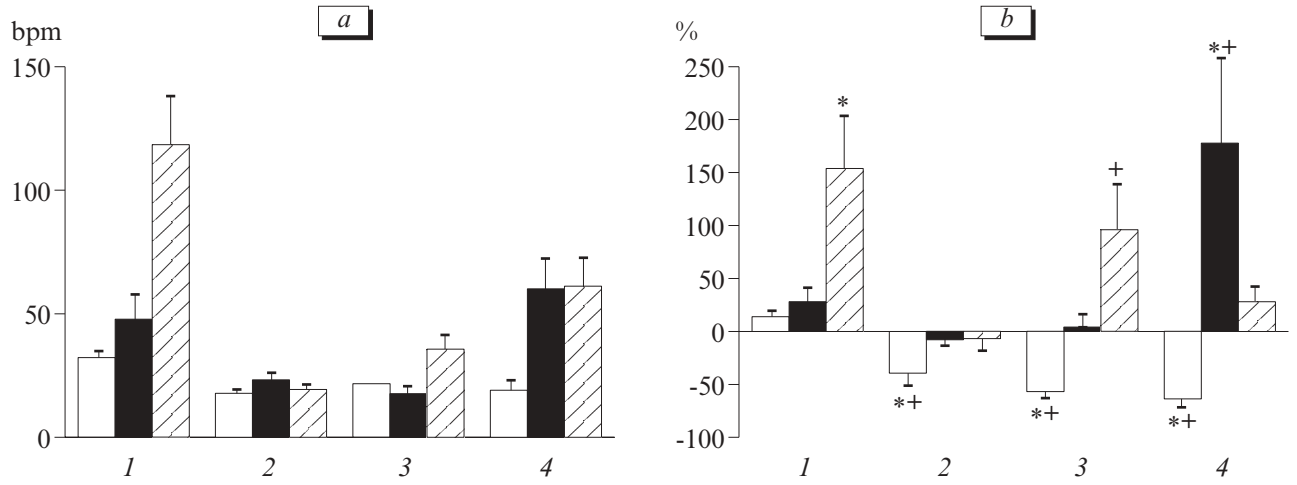

Fig. 1. Effects of endothelin-1 (ET-1) and Nω-nitro-L-arginine (L-NAME) on HR variability (standard deviation for 1000 cardiac cycles) in awake mice intravenously receiving hexamethonium, atropine, and atenolol: absolute values (*a*) and percentage (*b*). Light bars: NaCl. Dark bard: L-NAME. Shaded bars: L-NAME and ET-1. Control (*1*), hexamethonium (*2*), atropine (*3*), and atenolol (*4*). Significant differences ($p<0.05$, ANOVA-2) for *1*, *3*, and *4* (*a*). *Compared to baseline values (Wilcoxon *W* test); +compared to the corresponding control group (Mann—Whitney test).

β-adrenoceptor blockade with atenolol slightly increases this parameter [4]. In our experiments atropine and atenolol decreased HR variability. Blockade of NO synthase during administration of atropine had no effect on HR variability. It should be emphasized that ET-1 retained arrhythmogenic activity under these conditions. L-NAME sharply increased HR variability in mice receiving atenolol. The arrhythmogenic effect of ET-1 was blocked under these conditions. These changes were associated with an increase in parasympathetic tone during blockade of β-adrenoceptors. NO synthase blockade can enhance vagal influences on the heart, which sharply increases HR variability. Administration of L-NAME after treatment with atenolol primarily modulated neuronal NO synthase: BP did not increase under these conditions (4±1 *vs*. 17±2 mm Hg in the control, $p<0.05$).

Our results indicate that the autonomic nervous system plays an important role in the arrhythmogenic effects of ET-1 under conditions of NO synthase blockade. It is important that blockade of the sympathetic nervous system sharply increased HR variability induced by L-NAME. This effect was not observed during blockade of the parasympathetic nervous system. Arrhythmogenic activity of ET observed against the background of NO synthase blockade was preserved in animals receiving atropine, but disappeared after administration of atenolol.

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