Blockade of β-Adrenoceptors and Muscarinic Cholinergic Receptors Modulates Effect on Nitric Oxide on Heart Rate in Rats

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Nitroglycerine in doses of 0.4-1.0 mg/kg decreased the heart rate in rats, which was associated with inhibition of adrenergic influences realized via β-adrenoceptors. The negative chronotropic effect of sodium nitroprusside in a dose of 1 mg/kg was more significant compared to that of nitroglycerine (by 2-3 times). It was associated with inhibition of adrenergic and stimulation of cholinergic influences mediated via β-adrenoceptors and muscarinic cholinergic receptors, respectively. During blockade of β-adrenoceptors and muscarinic cholinergic receptors, sodium nitroprusside increased the time of atrioventricular conduction. These data indicate that function of myocytes in the heart conduction system of rats depends on the *PQ* interval.

Key Words: *nitric oxide; β-adrenoceptors; muscarinic cholinergic receptors; heart rate; PQ interval*

Nitric oxide (NO) donors nitroglycerine (NG) and sodium nitroprusside (SNP) produce not only a direct effect on the vascular endothelium (decrease blood pressure) [5], but also a dose-dependent negative chronotropic effect [3]. Activation of NO synthase (NOS) facilitates vagal nerve-mediated presynaptic transmission in cardiac ganglia [12]. At the postsynaptic level, cardiomyocyte eNOS modulates the response to muscarinic cholinergic stimulation. Probably, nNOS (in cardiac ganglions) and eNOS (in cardiomyocytes) potentiate vagal inhibition of the heart rate (HR). NO in physiological concentrations increases HR by activating the pacemaker current I_f [14], but in high concentrations produce an opposite effect [10]. It is difficult to differentiate the effects of NO on pacemaker cells, since this compound simultaneously affects target organs and produces different changes in spontaneous polarization (*e.g.*, inhibition of L-type Ca^{2+} current ($I_{Ca,L}$) and direct activation of pacemaker current I_f) [13]. *In vitro* experiments on cultured cardiomyocytes [11] and *in vivo* experiments on laboratory animals showed that NO suppresses the positive chronotropic responses to β-adrenoceptors (β-AR) agonists and sympathetic nerve stimulation [7]. NO blocks potential-dependent L-type Ca^{2+} channels under conditions of adrenergic stimulation [9]. Adrenergic influences and NO produce antagonistic effects on conduction and excitability, which plays a role in cardiac protection from arrhythmias [8]. Cardiomyocytes in eNOS knockout mice are characterized by low threshold for catecholamine-produced arrhythmias. Administration of a selective nNOS inhibitor increases the reaction of HR to sympathetic nerve stimulation in vagotomized animals [15]. The data indicate that neuronal NO *in vivo* potentiates this effect.

The relationship between adrenergic and cholinergic influences is impaired under certain conditions. The effects of NO donors under these conditions remains unknown.

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Here we studied the effects of NG and SNP in various doses on HR and *PQ* interval in rats upon blockade of β-AR and muscarinic cholinergic receptors (MCR).

MATERIALS AND METHODS

We studied the effects of nonselective β-AR antagonist propranolol (2 mg/kg) and nonselective MCR antagonist atropine (1 mg/kg) on modulation of HR and *PQ* interval in rats with NG and SNP. NG in increasing doses of 0.2, 0.4, 0.8, and 1.0 mg/kg was administered to rats of groups 1, 2, 3, and 4, respectively. Group 5 animals received SNP in a dose of 1 mg/kg (Sigma). In tables and figures, the reaction to the 1st treatment with NO donors is designated as NO1. The effect of treatment with NO donors under conditions of β-AR blockade is designated as NO2. The response to NO donors after blockade of MCR is designated as NO3. Experiments were performed on 53 adult male albino rats weighing 150-200 g and narcotized with 2 mg/kg chloral hydrate. ECG was recorded in standard lead II. On-line recording and processing of the data were performed using an L-264 analog-to-digital converter (Lcard) and original software. The results were analyzed by Student's *t* test.

RESULTS

NG (0.4-1.0 mg/kg) and SNP decreased HR in rats by 6.4-13.6% (Table 1, Fig. 1). HR returned to the baseline level after administration of NG in doses of 0.2 and 1.0 mg/kg. The effects of NG in other doses (0.4 and 0.8 mg/kg) and SNP (1.0 mg/kg) persisted for 6-8 min. SNP decreased HR by 27% (Fig. 1), which surpassed the negative inotropic effect of NG in different doses by 2-3 times.

β-AR antagonist decreased HR in NG-treated animals by 13.5-20.6% ($p<0.01$, Fig. 1). Propranolol less significantly decreased HR in rats receiving SNP (by 7.7%). NG in different doses had no effect, while SNP significantly decreased HR in animals with long-term blockade of β-AR (by 25.7%, $p<0.001$). Under these

TABLE 1. Effects of Propranolol and Atropine on Changes in HR in Rats Receiving NO Donors (bpm, *M*±*m*)

Conditions	NG, 0.2 mg/kg	NG, 0.4 mg/kg	NG, 0.8 mg/kg	NG, 1.0 mg/kg	SNP, 1.0 mg/kg
Baseline level	390.96±13.16	364.96±8.93	355.87±13.85	385.33±8.65	356.68±12.65
NO1	385.89±9.16	335.78±9.71*	307.50±10.15**	360.83±8.12*	260.41±11.97***
Baseline level	403.14±10.14	344.04±9.47	319.70±12.86	397.25±11.27	307.80±12.22
Propranolol	325.00±12.50***	292.28±8.02**	270.50±9.55***	315.27±11.13**	284.08±11.16
Baseline level	324.10±11.76	291.78±7.26	275.40±10.43	319.83±10.68	291.41±11.04
NO2	309.28±11.29	289.64±6.86	270.40±11.02	317.08±8.64	216.50±7.48***
Baseline level	308.78±10.17	284.85±5.72	275.90±10.56	327.83±14.54	279.50±10.15
Atropine	327.00±12.76	287.57±6.44	283.20±11.15	330.25±12.25	307.91±7.25*
Baseline level	320.14±12.83	287.00±6.52	293.10±11.47	333.08±13.40	307.91±12.20
NO3	319.28±12.85	286.15±7.01	291.10±10.72	321.80±9.26	255.08±9.65**

Note. Here and in Table 2: *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to baseline (before treatment).

TABLE 2. Effects of Propranolol and Atropine on Changes in the *PQ* Interval in Rats Receiving NO Donors (bpm, *M*±*m*)

Conditions	NG, 0.2 mg/kg	NG, 0.4 mg/kg	NG, 0.8 mg/kg	NG, 1.0 mg/kg	SNP, 1.0 mg/kg
Baseline level	34.29±2.06	37.51±1.93	38.87±2.93	33.50±2.82	36.82±3.09
NO1	35.00±2.32	43.57±2.04*	46.90±2.64*	35.50±2.77	47.75±3.74*
Baseline level	33.42±2.76	37.97±2.29	39.65±4.04	33.58±2.78	34.65±3.06
Propranolol	44.71±2.49**	49.92±1.46***	54.70±3.89*	43.99±3.03*	47.00±3.81*
Baseline level	42.72±2.77	43.78±3.49	45.60±3.05	44.75±3.20	45.30±3.47
NO2	46.78±2.44	52.64±1.28*	56.30±3.63*	44.33±3.78	55.30±3.42*
Baseline level	46.14±2.28	51.92±1.42	49.00±4.04	41.33±2.78	53.90±3.06
Atropine	42.07±3.84	51.14±1.26	46.33±3.06	37.90±2.79	48.20±3.81
Baseline level	45.21±2.79	51.92±1.26	46.33±3.05	37.90±2.36	49.50±3.05
NO3	45.42±3.02	52.07±1.28	51.00±3.02	48.40±3.08*	57.25±2.12*

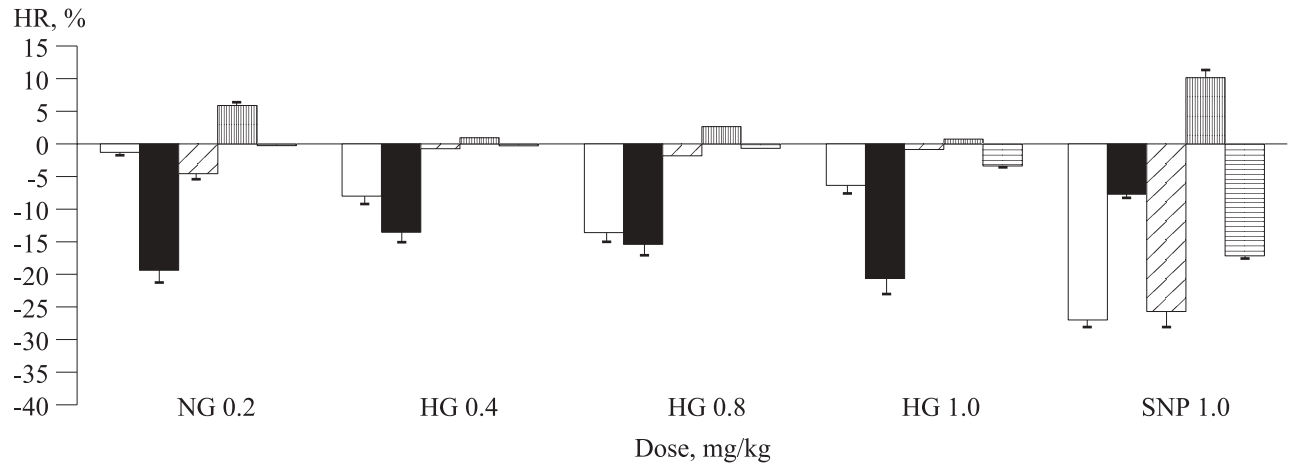

Fig. 1. Effect of propranolol and atropine on changes in HR produced by NO donors. Here and in Fig. 2: light bars, NO1; dark bars, propranolol; slant shading, NO2; vertical shading, atropine; horizontal shading, NO3.

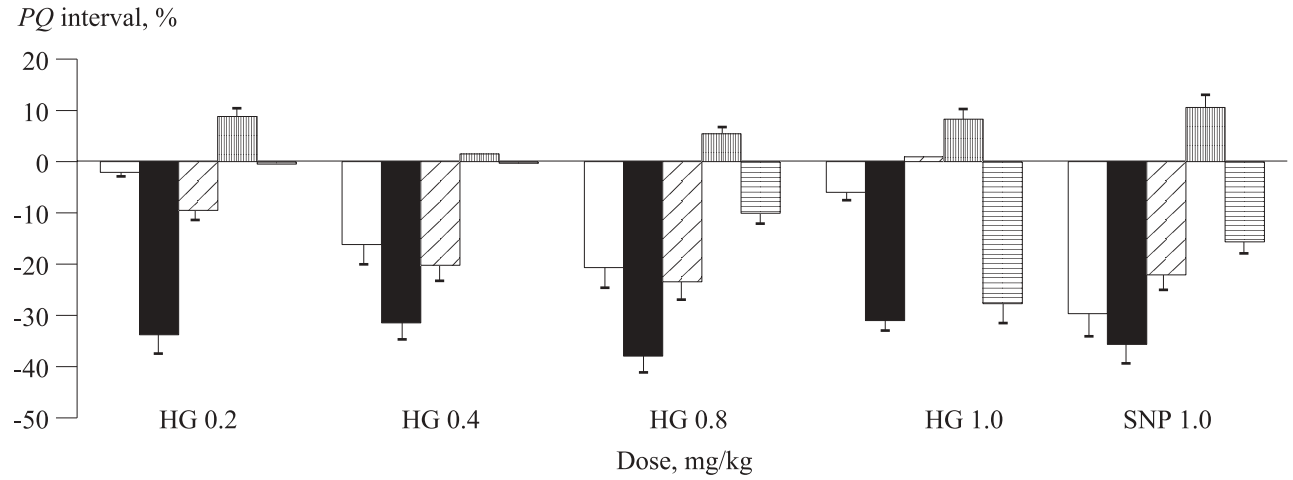

Fig. 2. Effect of propranolol and atropine on changes in *PQ* interval produced by NO donors.

conditions HR was minimum in all groups (216.5 bpm, Table 1).

In animals receiving NG in increasing doses atropine did not modulate HR, while SNP produced a positive chronotropic effect (by 10.2%, $p<0.05$). NG in various doses did not change HR under conditions of β-AR and MCR blockade. Under these conditions SNP in a dose of 1 mg/kg decreased HR by 17.2% ($p<0.01$, Fig. 1). Under conditions of β-AR blockade changes in HR were less significant compared to that produced by SNP (25.7%). NG (0.4 and 0.8 mg/kg) and SNP (1 mg/kg) decreased HR, which was probably associated with inhibition of adrenergic influences.

NG in doses of 0.2-1.0 mg/kg had no negative chronotropic effect during β-AR blockade. It can be hypothesized that the effect of NG is realized via suppression of β-adrenergic influences. Administration of NG in increasing doses under conditions of β-AR blockade had little effect on HR in rats with atropine-induced blockade of MCR. Further treatment with NG did not produce the negative chronotropic effect. Probably, the chronotropic effect of NG is not related to modulation of cholinergic influences.

Under conditions of β-AR blockade and SNP administration, blockade of MCR with atropine increased HR by 10.2% (Fig. 1). As differentiated from NG, SNP probably potentiates cholinergic modulation of HR. The negative chronotropic response to 1.0 mg/kg SNP persisted during blockade of β-AR and MCR. However, HR decreased less significantly under these conditions (by 17%). The negative chronotropic effect of SNP in a dose of 1.0 mg/kg was probably associated with inhibition of adrenergic and stimulation of cholinergic influences.

NO donors increased the *PQ* interval. These changes were most pronounced after administration of NG in doses of 0.4 and 0.8 mg/kg and SNP (Table 2). The *PQ* interval increased by 16, 21, and 30%, respectively (Fig. 2). We found that the *PQ* interval increases in animals of various groups during blockade of β-AR

with propranolol. Under these conditions the effect of NG depended on its dose. NG in doses of 0.2-0.8 mg/kg increased the *PQ* interval by 10, 20, and 23% ($p<0.05$).

The *PQ* interval increased in response to SNP administration during β-AR blockade (by 22.1%, $p<0.05$). NG in a dose of 1.0 mg/kg had no effect under these conditions (Fig. 2). During blockade of MCR with atropine administration of NG and SNP in a dose of 1.0 mg/kg increased the *PQ* interval by 27.7 ($p<0.05$) and 15.6%, respectively ($p<0.05$). These results suggest that SNP increases the *PQ* interval after subsequent blockade of β-AR and MCR.

The existence of NOS in various parts of the heart implies its involvement into modulation of cardiac function [10,14].

The hypotensive effect of NO donors (*e.g.*, NG) was studied in details. SNP is more potent than NG in stimulating NO production in rats [1]. SNP transformation is accompanied by the release of not only NO, but also of toxic cyanide [6]. SNP and NG decreased HR by 27 and 6.4%, respectively. Our results are consistent with published data that perfusion of rat heart with SNP decreased HR. The effect of SNP 4.6-fold exceeded that elicited by a substrate of NO synthesis L-arginine [2]. The negative chronotropic effect of SNP persisted for a long time.

NO donors modulate activity of cells in the cardiac conduction system and increase the time of atrioventricular conduction. NG and SNP prolong the *PQ* interval by 20.7 and 29%, respectively. We showed that SNP increases the *PQ* interval due to the inhibition of β-adrenergic influences.

NG in doses of 0.2-1.0 mg/kg produced no negative chronotropic effect under conditions of β-AR blockade. Therefore, this effect is associated with inhibition of β-adrenergic modulation of HR. The negative chronotropic effect of SNP is related to inhibition of adrenergic and stimulation of cholinergic influences mediated by β-AR and MCR, respectively. SNP increases the *PQ* interval by producing a direct effect on cells in the cardiac conduction system. The negative chronotropic effect of SNP was 2-3 times higher compared to that of NG.

The negative chronotropic effect of NO is probably mediated by presynaptic inhibition of norepinephrine release from sympathetic endings [11]. Neuronal NO potentiates vagal bradycardia via presynaptic modulation of neurotransmission [7]. This compound increases the release of acetylcholine. NO synthesized in cells of mammalian sinoatrial node is involved in cholinergic modulation of HR. In nerve cells, NOS coexists with other traditional neurotransmitters and neuropeptides. This enzyme is most frequently detected in cholinergic neurons [4].

Our findings suggest that the cardiac response to adrenergic stimulation is inhibited by the NOergic system. The mechanisms underlying the *in vivo* inhibitory effect of NO on the heart include modulation of calcium inward current through L-type Ca^{2+} channels, presynaptic inhibition of norepinephrine release from sympathetic nerve endings, and facilitation of acetylcholine secretion from vagal nerves.

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