Nitric Oxide Donors Dose-Dependently Reduce Heart Rate in Rats against the Background of Blood Pressure Drop

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The effect of nitroglycerin in increasing doses (0.2-1.0 mg/kg) and sodium nitroprusside (1 mg/kg) on the heart rate, *P-Q* interval of ECG, and blood pressure was studied on 53 albino rats. NO donors nitroglycerin and sodium nitroprusside directly affected vascular endothelium (the maximum decrease in blood pressure was 28.5 and 57.0%, respectively) and produced a dose-dependent negative chronotropic effect (the maximum decrease in heart rate was 13.6 and 27.0%, respectively). These drugs also affected myocytes of the cardiac conduction system: *P-Q* interval increased by 16.0-29.7%.

Key Words: *nitroglycerin; sodium nitroprusside; heart rate; P-Q interval; blood pressure*

Nitric oxide (NO) is an important modulator of cardiac function, because of the presence of NO-synthases in all cardiac subdivisions and pronounced effects of NO on vascular tone and reactivity, blood pressure (BP), and cardiac parameters [8,12]. NO released from the coronary epithelium, endocardium, and cardiomyocytes participates in functional regulation of the heart [8]. In mammals, NO synthesized by cells of the sinoatrial node contributes to the cholinergic control of the heart rate (HR) [7]. Increased content of NO was found in children with cardiac rhythm disturbances and in young athletes with partial right bundle-branch block and migration of the pacemaker [2]. Both organic nitrate nitroglycerin (NG) and inorganic nitric compound sodium nitroprusside (SNP), the exogenous NO donors, are widely used in clinical practice as hypotensive preparations. However, there are no data on the dose-dependent effect of various NO donors on atypical myocytes of the cardiac conduction system.

Our aim was to compare the effects of various doses of NG and SNP on HR, *P-Q* interval and *QRS* complex on ECG, and BP in rats.

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MATERIALS AND METHODS

Experiments were carried out on mature male albino rats (n=53) weighing 150-200 g anesthetized with chloral hydrate (2 mg/kg). BP in the femoral artery was measured with an MLT0698 pressure transducer (ADInstruments). ECG was recorded in standard lead II using an EK1T-03M2 cardiograph. The data were processed on-line using an L-264 digitizer (Lcard, Moscow) and original software. ECG and BP were recorded throughout the experiment. Groups 1-4 rats received NG in doses of 0.2, 0.4, 0.8, and 1.0 mg/kg, respectively. Group 5 rats received 1 mg/kg SNP (Sigma). The data were processed statistically using Student's t test.

RESULTS

NG in a dose of 0.2 mg/kg had no effect on HR (Table 1), although in a dose of 0.4 mg/kg it decreased HR by 29.0 bpm on postinjection minute 2 (p<0.05). NG administered in a dose of 0.8 mg/kg produced more pronounced bradycardia (HR decreased by 48.3 bpm). In this case the effect was observed 1 min postinjection. NG in a dose of 1.0 mg/kg produced only a tran-

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sient decrease in HR (by 24.5 bpm). Thus, NG had no effect on HR in a dose of 0.2 mg/kg and induced bradycardia in doses of 0.4 and 0.8 mg/kg (by 29 and 48 bpm, respectively). Paradoxically, higher doses produced a less pronounced chronotropic effect.

In experiments with SNP, a decrease in HR was observed on postinjection minute 1 and was most pronounced (96.3 bpm) after 3 min. In SNP-treated rats HR decreased to 260.4 bpm. The maximum negative chronotropic effect of SNP 4-fold surpassed that of NG in the corresponding dose. NG produced the maximum negative chronotropic effect in a dose of 0.8 mg/kg, but cardiac response to 0.8 mg/kg NG was 2-fold less pronounced than that to SNP. Thus, NO donors produced a dose-dependent negative chronotropic effect in rats and the effect of SNP 2-4-fold surpassed that of NG.

The *P-Q* interval on ECG characterizes the time of atrioventricular conduction. We observed no significant changes in this parameter after injection of 0.2 mg/kg NG. NG in a dose of 0.4 mg/kg increased this parameter by 6.06 msec on minute 3 postinjection (Table 2). When NG was injected in a dose of 0.8 mg/kg, the duration of *P-Q* interval increased by 8.08 msec on minute 2. The maximum increase in this parameter (to 47.75 msec) was observed on minute 2.5 postinjection ($p<0.05$).

After injection of SNP the length of *P-Q* interval was greater by 13.5 msec than after administration of NG in the corresponding dose ($p<0.05$). Therefore, NG and, particularly, SNP inhibit atrioventricular conduction, which means that they directly affect not only atypical cardiomyocytes in the sinus node as indicated by HR drop, but also cardiomyocytes in the distal portions of the conduction system. We reveled a tendency to lengthening the QRS complex after injection of NG or SNP. This effect varied in different groups, al-

TABLE 1. Effect of Various Doses of HG and SNP on HR (bpm) in Rats (*M*±*m*)

Time postinjection, min	NG, mg/kg				SNP, 1 mg/kg
	0.2	0.4	0.8	1.0	
Control	390.96±13.16	364.96±8.93	355.87±15.85	385.33±8.65	356.68±12.65
0.5	402.86±8.95	355.57±8.98	340.30±16.20	400.00±10.21	334.00±16.08
1	401.50±15.23	340.42±11.31	312.70±14.20*	382.91±11.32	293.16±11.77*
1.5	398.05±15.74	340.92±9.74	309.90±14.21*	360.83±8.12*	275.58±7.39*
2	395.36±16.24	335.92±10.95*	307.50±10.15*	377.58±11.20	271.33±9.27*
2.5	394.07±16.89	335.78±10.71*	308.10±14.22*	382.33±10.11	265.83±10.54*
3	394.21±16.34	336.28±10.77*	309.50±14.64*	384.16±11.32	260.41±11.97*
3.5	396.21±9.23	337.78±10.91*	311.30±15.60	389.16±11.54	275.75±15.73*
4	398.57±8.74	336.50±10.62*	313.50±16.72	391.16±15.76	276.58±14.29*
4.5	403.14±10.14	344.04±9.47	319.70±14.86	397.25±14.27	307.80±18.42*

Note. Here and in Tables 2 and 3: *$p<0.05$ compared to the control.

TABLE 2. Effect of Various Doses of HG and SNP on *P-Q* Interval (msec) in Rats (*M*±*m*)

Time postinjection, min	NG, mg/kg				SNP, 1 mg/kg
	0.2	0.4	0.8	1.0	
Control	34.29±2.06	37.51±1.93	38.87±2.93	33.50±2.82	36.82±3.09
0.5	33.30±2.02	33.64±2.68	38.94±3.05	30.00±3.28	38.16±3.74
1	30.64±2.14	39.42±1.81	44.87±3.05	32.08±2.61	42.50±3.04
1.5	31.64±2.49	41.35±2.76	45.87±3.07	31.00±2.86	41.75±3.26
2	30.71±1.92	42.78±2.32	46.90±2.64*	33.41±2.92	45.91±3.01*
2.5	33.50±2.03	41.78±3.16	42.90±3.65	34.25±2.78	47.75±3.74*
3	35.07±2.75	43.57±2.04*	44.30±3.02	35.50±2.77	40.00±3.53
3.5	35.00±2.33	38.28±2.65	45.30±3.97	31.08±2.12	34.58±3.81
4	34.50±3.11	41.14±2.29	43.70±3.57	34.50±4.24	40.16±3.81
4.5	33.42±2.76	37.97±2.29	43.00±3.04	33.58±3.14	32.65±3.37

though the differences from the control were insignificant.

Both the NG- and SNP-induced drops in systolic pressure were observed as early as 30 sec postinjection (Table 3). After increasing NG dose, the reaction increased from 14.64 to 23.57 mm Hg ($p<0.05$). SNP produced even more pronounced drop in systolic pressure (by 34.52 mm Hg, $p<0.05$). It should be noted that the effect of NG rapidly disappeared, while SNP-induce drop in systolic pressure of 38.34 mm Hg persisted to postinjection minute 2, after which systolic pressure returned to the initial level on minutes 4-5.

The maximum decrease in systolic pressure produced by SNP (by 50.76 mm Hg) was 2 times greater than that of NG. NG produced a dose-dependent drop in diastolic pressure by 10.86-17.69 mm Hg, and then diastolic pressure rapidly recovered. By contrast, SNP-induced drop in diastolic pressure (by 45.07 mm Hg) persisted to postinjection minute 2. The diastolic pressure decreased during following 2 min and then restored to the initial value. Therefore, NG exerted the maximum hypotensive effect in a dose of 1 mg/kg. When used in the same dose, SNP induced a more pronounced and longer drop in BP.

The hypotensive effect of NO donors is widely known. In our experiments this action was revealed as early as 30 sec postinjection. The drop in systolic pressure was dose-dependent: increasing NG dose from 0.2 to 1.0 mg/kg corresponded to an increase in the reaction from 16.4 to 28.5%. The duration of the hypotensive effect induced by NG in doses of 0.2-0.8 mg/kg was less than 30 sec. When NG dose was 1.0 mg/kg, the hypotensive effect persisted for 2.5 min. SNP demonstrated much greater effect on systolic pressure: it decreased it by 57% on postinjection minute 2. Moreover, the hypotensive effect of SNP lasted for a longer period (up to 4 min). We also observed a 15.5-24.2% drop in diastolic pressure in response to increasing doses of NG and its decrease by 60% after injection of SNP. By contrast, L-NAME, a blocker of eNO-synthase, increased BP in rats by 22% [13]. It is assumed that other isoforms of NO-synthase are not involved in BP control, but they can participate in the control of HR.

The dynamics of the effects of NO donors varied: the low doses of NG decreased BP for a short time, while bradycardia developed after BP recovery. Simultaneously, changes in the duration of *P-Q* interval occurred. These data attest to the existence of different independent mechanisms of the effect of NO donors on vascular system and the heart. L-NAME provoked bradycardia both in control mice and in mice whose eNO-synthase was knocked out indicating that eNO-synthase and other forms of NO-synthase could be involved in HR control [9]. A reflectiry rise of HR should be expected in response to pronounced hypotensive action of NG and SNP. Paradoxically, we revealed an opposite effect. While NG produced no marked changes in HR when applied in a dose of 0.2 mg/kg, it evoked a negative chronotropic effect (8%) in a dose of 0.4 mg/kg. Even more pronounced bradycardia of 12.0-13.6% was observed after injection of NG in a dose of 0.8 mg/kg. However, further increase of NG dose to 1 mg/kg produced less pronounced bradycardia: HR dropped only by 6.4%. There is evidence that NO applied in physiological concentrations increased HR, while higher doses of NO decreased it [9].

In a dose of 1 mg/kg SNP decreased HR by 27%, while the same dose of NG produced only a 6.4% drop in HR. Perfusion of the heart with SNP produced an inhibitory effect on HR, which 4.6-fold surpassed that of L-arginine [3]. The negative chronotropic effect of SNP persisted for a long time.

TABLE 3. Effect of Various Doses of HG and SNP on Systolic Pressure (mm Hg) in Rats (*M±m*)

Time postinjection, min	NG, mg/kg				SNP, 1 mg/kg
	0.2	0.4	0.8	1.0	
Control	89.34±3.58	91.55±2.48	94.24±5.31	81.68±4.91	89.10±5.00
0.5	74.70±6.03*	69.78±6.09*	70.67±6.23*	60.63±8.95*	54.58±12.51*
1	84.47±6.14	77.15±7.13	86.89±6.99	58.43±6.03*	45.69±12.43*
1.5	87.05±5.74	86.39±3.31	87.30±6.60	60.95±7.24*	42.97±11.02*
2	88.98±5.33	88.57±3.11	89.04±6.15	61.06±8.71*	38.34±7.25*
2.5	90.22±4.65	89.58±2.81	90.35±6.41	61.86±7.41*	40.68±7.31*
3	91.84±4.46	90.55±1.91	90.90±6.68	62.31±9.32	42.39±9.15*
3.5	92.78±4.09	91.53±1.73	91.35±6.33	63.45±8.67	46.13±8.37*
4	93.17±3.90	92.39±1.69	91.79±5.52	63.21±8.76	47.05±8.26*
4.5	94.37±1.12	93.12±1.96	92.03±3.80	75.90±6.63	81.12±2.46

Much evidence points to the effect of NO on various functions in mammals and amphibia [1,5], cardiac chronotropic function included [4,6,11,14]. SNP potentiates vagus-induced bradycardia in mouse atria [6]. NO plays a key role in the regulation of HR in dogs [11]. Cardiomyocytes express endothelial and inducible NO-synthase. The cardioinhibitory effect of endogenous NO can play a role in some forms of cardiac insufficiency [9]. HPLC showed that profobol upregulates NO synthesis in cultured cardiomyocytes from rat ventricles by 80%, but produced a negative chronotropic effect on the heart [14].

In physiological concentrations NO increases HR via activation of hyperpolarization-induced inward current, but this effect is less pronounced at high concentrations of NO [9]. Similar to the effect produced by activation of K_{ATP} channels, NO inhibits peripheral sympathetic activity in the heart [10], which probably underlie its effects on HR. NO donors also affect myocytes of the cardiac conduction system and inhibit atrioventricular conduction, which is seen from lengthening of *P-Q* interval by 16.2 and 20.7% after injection of NG. Even more pronounced increase of this interval (24.7% and maximum 29.7%) was observed after injection of SNP.

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