Sodium Nitroprusside Increases Pacemaker Rhythm of Sinoatrial Nodes via Nitric Oxide-cGMP Pathway

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Abstract

Effects of sodium nitroprusside (SNP), a nitric oxide donor, on the action potential in isolated guinea-pig sinoatrial nodes and ventricular papillary muscles were investigated. In the driven ventricular papillary muscle, SNP (10^{-10}–10^{-3} M) decreased the twitch tension in a concentration-dependent manner without significantly changing the configuration of action potential and the maximal velocity of depolarizing upstroke. In isolated sinoatrial nodes, SNP (10^{-8} - 10^{-3} M) increased the pacemaker rhythm in a concentration-dependent manner. At 10^{-5} M SNP, the pacemaker activity increased from 197.2± 6.1 to 221.4±9.7 bpm. Changes of configuration of the action potential included a decrease of the duration of repolarization, i.e., from peak to the maximal diastolic potential (MDP), from 141.4±6.4 to 130.0±7.0 ms and an increase of the slope of the diastolic membrane potential from 101.6±5.3 to 116.5± 7.3 mV/s (n=6, $p<0.05$). However, MDP and threshold potential were not significantly changed. Methylene blue (MB, 10^{-5} M), a guanylate cyclase inhibitor, significantly decreased the pacemaker activity of the sinoatrial node by increasing the durations of repolarization and diastolic depolarization. After pretreatment with 10^{-5} M MB, the effect of SNP was inhibited. The results indicate that nitric oxide, released from SNP, increases the pacemaker activity by enhancing the rates of repolarization and diastolic depolarization. These effects are possibly due to increases in delayed-rectifier K^+ and diastolic slow inward currents, which are involved in a mechanism associated with the NO-cGMP pathway.

Key Words: methylene blue, pacemaker potential, sinoatrial node, funny current, potassium current, action potential

Introduction

Sodium nitroprusside (SNP) is widely used to lower the arterial blood pressure in clinical or basic cardiovascular studies. Previous studies have shown that SNP can be readily metabolized to nitric oxide (NO) in subcellular fractions (12). It is also known that NO can activate guanylate cyclase to increase intracellular cGMP which can efficiently decrease the vascular smooth muscle tone causing a fall of arterial blood pressure (3). Although an increase in heart rate can be mediated by the arterial baroreflex, SNP can also increase heart rate in transplant heart and the beat rate of isolated sinoatrial preparation (4, 7, 14, 21). In cardiac myocytes, NO can regulate both adenylate cyclase and guanylate cyclase (19). The production of NO, which acts as a mediator, is essential for the modulation of myocardial contractility and membrane currents (8, 11). But the exact mechanism by which elevations in NO elicit the electromechanical effects on the cardiac tissues is still controversial (2, 11, 16, 17, 19).

The present work was undertaken to investigate whether SNP could directly affect the action potentials

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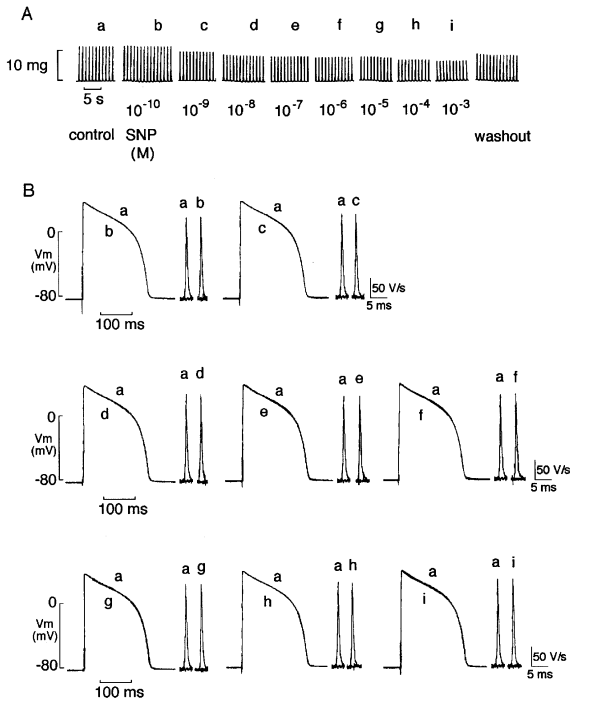

Fig. 1. Effects of SNP on twitch tension and action potential in a ventricular papillary muscle. SNP was cumulatively added. A. Twitch tensions, vertical and horizontal bars indicating twitch force and time, respectively. Recordings were taken after exposure to each concentration of SNP for 15 min. B. The action potentials (b to i) were taken and superimposed on the control (a) after exposure to each concentration of SNP for 15 min, as indicated in A. The $\dot{V}_{max}$ of the action potential was indicated at right of the action potential.

of the isolated sinoatrial node (SA node) preparation and the ventricular papillary muscle of guinea pig hearts. Changes in the configuration of the action potential were analyzed. We found that SNP induced a concentration-dependent negative inotropic response without significant changes of action potential in the ventricular papillary muscle and produced a positive chronotropic response in SA node cells. The increase in the pacemaker activity was associated with enhancements of repolarization and slow diastolic depolarization of the action potential. This effect could be inhibited by MB, a guanylate cyclase inhibitor, suggesting that the increase in pacemaker activity after SNP was due to stimulation of delayed-rectifier K^+ and depolarizing pacemaker currents via the NO-cGMP pathway.

Materials and Methods

Male guinea pigs, weighing 250-450 g, were sacrificed after anesthetized with sodium pentobarbital (40 mg/Kg, ip). The heart was quickly removed and soaked in preoxygenated normal Tyrode's solution. A sinoatrial node/atrial preparation, roughly 3 mm in diameter and 5 mm in length, near the superior vena cava, was carefully dissected from the right atria and mounted in a narrow perfusion chamber (0.2 ml) (1). Also, a papillary muscle, 1 mm in diameter and 3 mm in length, was dissected from the right ventricle of the heart. The papillary muscle was mounted in a narrow perfusion chamber and was driven with a Grass stimulator at 60 bpm (20). The twitch tension was measured by a force displacement transducer (Cambridge 403A) and recorded on a chart recorder (Gould). The muscle fiber was stretched to 70 % of the maximal twitch tension. The flow rate of the perfusate was maintained at 8 ml/min. The composition of the perfusate was (in mM): NaCl 137.0; KCl 5.4; $CaCl_2$ 1.8; $MgCl_2$ 1.1; NaH_2PO_4 0.5; $NaHCO_3$ 12; Glucose 5.0; and was equilibrated with 97 % O_2 and 3 % CO_2 gas giving a pH of 7.36. The temperature of the chamber fluid was maintained at 36 °C.

Conventional microelectrodes were pulled from borosilicate glass (Corning 7740) with tips smaller than 1 μ and resistance ranged from 10 to 40 MΩ when backfilled with 3 M KCl solution. A digital storage oscilloscope (Gould Model 1604) recorded the action potential. The maximal rate of depolarization of the action potential ($\dot{V}_{max}$) was recorded after the signal from the conventional microelectrode passed through a differential amplifier. At least two hours were allowed for equilibration before the beginning of the experiment. Sodium nitroprusside (SNP) and methylene blue (MB) were purchased from Sigma Chemical Company.

The data were presented as means±standard error of the mean. An ANOVA or a Student's t test was employed for analyzing the experimental data. The difference of the means was considered significant when the p value was less than 0.05.

Results

Effects of SNP on Ventricular Papillary Muscles

The effects of SNP on the action potential and twitch tension of the ventricular papillary muscle are shown in Figure 1. It can be seen from this figure, SNP from 10^{-10}-10^{-3} M decreased the contractile force in a concentration-dependent manner, but had no significant effect on the action potential. In 8 papillary muscle fibers, the amplitude (APA), durations at 30% and 90% repolarization (APD_{30} and APD_{90}), and $\dot{V}_{max}$ of the action potential at control were 119.3±0.3 mV, 146.5±2.1 ms, 195.4±1.9 ms, and 202.6±3.7 V/s, respectively. None showed significant change when SNP was added from 10^{-10} to 10^{-3} M. The twitch tension, however, decreased in concentration-dependent manner and reduced by 27 % of control after 10^{-3} M SNP (Fig. 2).

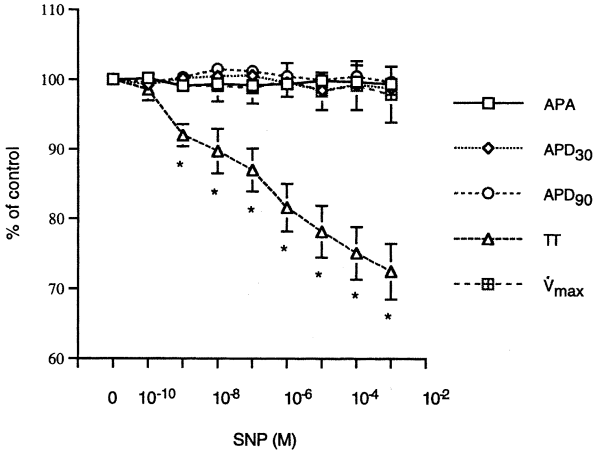

Fig. 2. Mean data for 8 experiments showing the effects of SNP on the action potential and twitch tension (TT) in ventricular papillary muscles. The control values of amplitude (APA), durations at 30% and 90% repolarization (APD_{30} and APD_{90}), and maximal rate of depolarization ($\dot{V}_{max}$) of the action potential were 119. 3±0.3 mV, 146.5±2.1 ms, 195.4±1.9 ms, and 202.6±3.7 V/s, respectively. *: $p<0.05$ as compared to control.

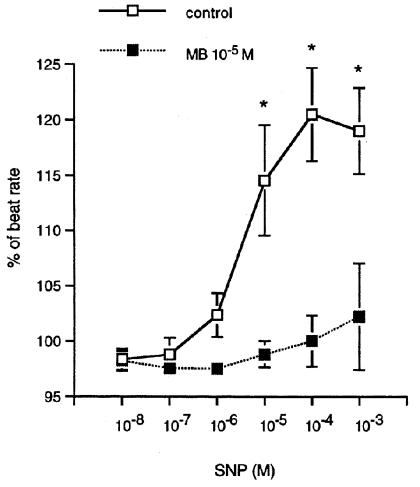

Fig. 3. Concentration-dependent effects of SNP on the pacemaker rhythm of SA nodes. ❑: SNP only (n=7); ■: SNP after pretreatment with 10^{-5} M MB (n=5). *: $p<0.05$ as compared to control.

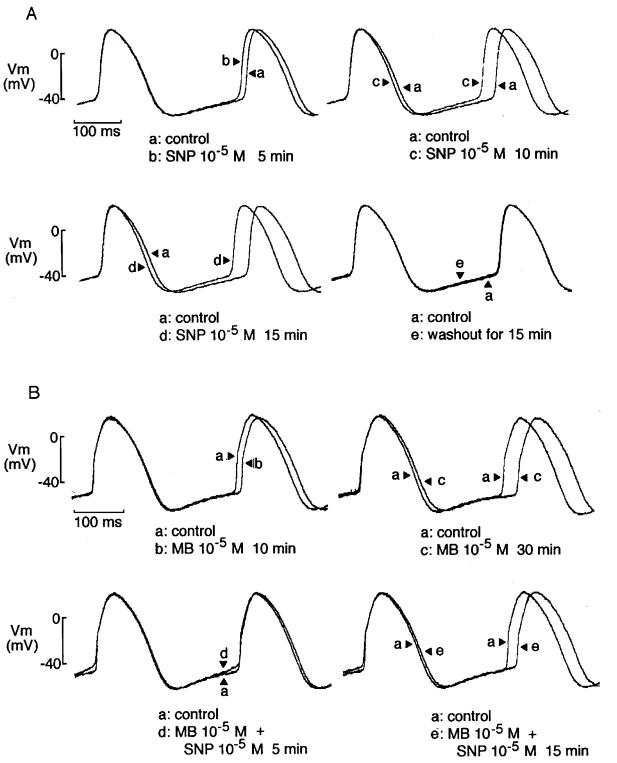

Fig. 4. A. Effects of SNP on the action potential of a SA nodal cell. The action potential (a) was recorded as control when the SA node was stabilized for about two hours. The superimposed action potentials (b), (c), (d) were taken at 5, 10, 15 min, respectively, after 10^{-5} M SNP was added. The action potential recovered in 15 min when SNP was deleted from the bath (action potential e). B. Effects of MB and SNP on the action potential of a SA nodal cell. The action potential (a) was recorded as control after the SA node was stabilized for two hours. Action potentials (b) and (c) were taken after exposure to 10^{-5} M MB for 10 and 30 min, respectively. In the superimposed action potentials, MB prolonged the durations of repolarization and diastolic depolarization as well as the cycle length of the pacemaker activity. After pretreatment with 10^{-5} M MB, the addition of 10^{-5} M SNP produced a positive chronotropy and enhanced the repolarization and the diastolic depolarization in 5 min (action potential d). The positive chronotropic response was eventually inhibited after exposure for 15 min (action potential e).

Effects of SNP and MB on SA nodes

The concentration-dependent effect of SNP on the pacemaker rhythm of SA nodes is shown in Figure 3. As shown in the figure, the positive chronotropic response became maximal with SNP concentration at 10^{-4} M. This chronotropic effect could be inhibited after pretreatment of the fiber with 10^{-5} M MB. Figure 4 shows typical changes of the action potential. In this figure, 10^{-5} M SNP progressively shortened the cycle length of the pacemaker action potential. During exposure, the slope of diastolic depolarization increased in the first 5 minutes, and the repolarizing duration from peak of the action potential to maximal diastolic potential (MDP) decreased to a steady state in 15 minutes. These changes could be completely reversed after SNP was washed out (Fig. 4A). In 6 SA nodes, 10^{-5} M SNP increased the pacemaker rhythm by nearly 14.2 %. The duration of repolarization significantly decreased from 141.4±6.4 to 130.0±7.0 ms, or about 7.8%, and the slope of diastolic depolarization significantly increased from 101.6± 5.3 to 116.5±7.3 mV/s. MDP and threshold potential (TP) were not significantly changed (Table 1). On the other hand, 10^{-5} M MB increased the cycle length of the action potential. Durations of repolarization and diastolic depolarization were prolonged (Fig. 4B). In 5 SA nodes, 10^{-5} M MB decreased the pacemaker rhythm from 198.0±6.5 to 182.9±4.9 bpm, nearly

Table 1. Effects of Sodium Nitroprusside (SNP) and Methylene Blue (MB) on the Action Potential of the Sinoatrial Node of Guinea Pigs

	n	Rhythm (bpm)	RT (ms)	MDP (mV)	TP (mV)	DDS (mV/s)
Control	6	197.2±6.1	141.4±6.4	-63.1±4.1	-50.0±4.6	101.6±5.3
SNP 10^{-5} M						
5 min		206.2±7.2*	138.3±5.0	-63.0±4.2	-50.0±4.8	107.7±6.0
10 min		217.2±9.3*	130.0±6.3*	-63.6±4.2	-50.6±4.8	113.5±5.5*
15 min		221.4±9.7*	130.0±7.0*	-63.1±4.2	-50.4±4.7	116.5±7.3*
Washout		196.8±6.1	141.9±5.9	-63.3±3.6	-50.1±4.6	94.5±4.2
Control	5	198.0±6.5	134.0±5.1	-59.9±3.6	-44.9±3.2	127.1±9.3
MB 10^{-5} M						
10 min		194.1±5.8	136.7±5.0	-59.9±3.5	-44.9±3.1	122.4±4.7
30 min		182.9±4.9*	141.3±3.5*	-59.9±3.3	-45.7±2.6	113.8±7.3*
+SNP 10^{-5} M						
5 min		190.5±8.7	140.3±6.4	-61.6±2.7	-45.6±2.6	137.1±13.4
15 min		186.7±6.8*	141.0±4.1*	-61.3±2.9	-45.9±2.5	117.2±8.3*

Data were presented as mean±SEM. Abbreviations: bpm, beats/min; RT, repolarizing time from the peak to the maximal diastolic potential; MDP, maximal diastolic potential; TP, threshold potential; DDS, slope of diastolic depolarization. *: $p<0.05$ as compared to control value.

7.5%, in 30 min. The duration of repolarization increased significantly from 134.0±5.1 to 141.3±3.5 ms and the slope of diastolic depolarization decreased significantly from 127.1±9.3 to 113.8±7.3 mV/s. After pretreatment with 10^{-5} M MB, effects of 10^{-5} M SNP were significantly inhibited except for transient increases in pacemaker activity and slope of diastolic depolarization (Fig. 4B and Table 1).

Discussion

In the present study, we have demonstrated that SNP accelerates the heart rate in a concentration-dependent fashion. The positive chronotropic effect can be inhibited by MB, indicating a mechanism involving the NO-cGMP pathway. In the ventricular papillary muscle, SNP decreases the contractile force without significantly changing the action potential even in rather high concentrations. But, in the sinoatrial node, SNP significantly increases the rates of repolarization and diastolic depolarization. It thus appears that distinctly different ionic channels at the pacemaker cells and at the ventricular muscles are selectively intervened by NO.

From recent reports, it is controversial regarding the direct inotropic action of NO donors in cardiac tissues (2, 5). NO donors have been shown to have little effect on cardiac Ca^{2+} currents in cardiomyocytes under basal condition (8, 11). SNP is shown to exert a negative inotropic effect on atrial and ventricular myocardium via generation of cGMP (2). On the other hand, 3-morpholinosydnonimine (SIN-1), another type of NO donor, has been shown to enhance the contractility and cardiac Ca^{2+} currents, in concentrations at micromolar ranges (2, 10). The underlying mechanism is interpreted to involve a complex subcellular reaction through the NO-cGMP pathway or a non-cGMP-mediated pathway (7, 14). In the present study, we found that SNP decreased the contractile force but did not significantly change the configuration of the action potential in the ventricular papillary muscle, even in millimolar concentrations, suggesting that SNP primarily exerted an effect on the contractile event rather than on ionic currents across the membrane in the guinea pig ventricular papillary muscle. The decreased twitch tension can be due to a PKG-dependent reduction in myofilament responsiveness to Ca^{2+} in the modulation of NO (2, 19).

In the sinoatrial node, alteration of the pacemaker activity can be achieved by changing the rate of repolarization, the level of maximal diastolic or threshold potential, and/or the slope of diastolic depolarization. It is known that the repolarizing phase of the action potential is mainly brought about by activation of delayed rectifier K^+ channels (15, 18). The diastolic depolarization in maintaining pacemaker activity is generated by the slow inward current, which is contributed from the sustained inward current (I_{st}), hyperpolarization-activated inward currents (I_f) and decay of K^+ currents (6, 15, 18). Recent studies have shown that NO donors increase

the pacemaker activity by enhancing the hyperpolarization-activated inward current in SA nodal cells of rabbit or guinea pig hearts (9, 14). In sinoatrial preparations of guinea-pig hearts, external application of a membrane-permeable analogue of cGMP, 8-Br-cGMP, increases the beating rate at high concentrations (14). In the present study, SNP significantly increased the slope of the diastolic depolarization and the rate of repolarization without changes in the MDP, TP, and amplitude of the spike. The shortening of the repolarization duration suggests that the delayed rectifier K^+ current, main outward currents during the repolarization, was increased (6). The increased slope of diastolic depolarization indicates that the slow inward current, most likely I_f, was enhanced (21). Therefore, the positive chronotropic response could be attributed to the stimulatory effects of NO, released from SNP, on repolarization and depolarization. Since MB, a guanylate cyclase inhibitor, could inhibit all these effects, the positive chronotropic response induced by SNP appears to involve a mechanism associated with the NO-cGMP signal pathway (21).

We also demonstrated in the present study that MB can directly inhibit the pacemaker rhythm by prolonging the times for repolarization and depolarization and pretreatment with MB could not abolish the initial increase in the pacemaker rhythm in response to SNP. In an early biochemical study, MB was shown to inhibit cellular soluble guanylate cyclase and NO synthase (13). Therefore, the direct inhibitory effect of MB could be attributed to decreases in intracellular cGMP and NO content. A transient positive chronotropic effect of SNP after pretreatment with MB for 30 minutes suggests a non-cGMP-mediated action of SNP and an important role of basal endogenous NO production in maintenance of heart rate. From the reported data, the cGMP-independent positive chronotropic effect might be due to the generation of superoxide anion or the nitrosylaion of some regulatory protein in cells (8). A discussion of the exact mechanism(s) is beyond the scope of the present study.

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