Circulation Research

(*Circulation Research.* 1997;81:60-68.)

Articles

Nitric Oxide Can Increase Heart Rate by Stimulating the Hyperpolarization-Activated Inward Current, I_f

Piotr Musialek, Ming Lei, Hilary F. Brown, David J. Paterson, , Barbara Casadei

From the University Laboratory of Physiology (P.M., M.L., H.F.B., D.J.P.), Oxford, UK, and the Department of Cardiovascular Medicine (P.M., B.C.), John Radcliffe Hospital, Oxford, UK.

Correspondence to Piotr Musialek, MD, Department of Cardiovascular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK. E-mail piotr.musialek@clinical-medicine.ox.ac.uk

Abstract

Abstract We investigated the chronotropic effect of increasing concentrations of sodium nitroprusside (SNP, n=8) or 3-morpholinosydnonimine (SIN-1, n=6) in isolated guinea pig spontaneously beating sinoatrial node/atrial preparations. Low concentrations of NO donors (nanomolar to micromolar) gradually increased the beating rate, whereas high (millimolar) concentrations decreased it. The increase in rate was (1) enhanced by superoxide dismutase (50 to 100 U/mL, n=6), (2) prevented by the guanylyl cyclase inhibitors 6-anilino-5,8-quinolinedione (5 μmol/L, n=6) or 1*H*-(1,2,4)oxadiazolo(4,3-*a*)quinoxalin-1-one (10 μmol/L, n=6), and (3) mimicked by 8-bromo-cGMP (n=6) with no additional positive chronotropic effect of SIN-1 (n=5). The response to 10 μmol/L SNP (n=28) or 50 μmol/L SIN-1 (n=16) was unaffected by I_{Ca-L} antagonism with nifedipine (0.2 μmol/L) but was abolished after blockade of the hyperpolarization-activated inward current (I_f) by Cs^+ (2 mmol/L) or 4-(*N*-ethyl-*N*-phenylamino)-1,2-dimethyl-6-(methylamino)pyrimidinium chloride (1 μmol/L). The effect on I_f was further evaluated in rabbit isolated patch-clamped sinoatrial node cells (n=21), where we found that 5 μmol/L SNP or SIN-1 caused a reversible Cs^+-sensitive increase in this current (+130% at -70 mV and +250% at -100 mV). In conclusion, NO donors can affect pacemaker activity in a concentration-dependent biphasic fashion. Our results indicate that the increase in beating rate is due to stimulation of I_f via the NO-cGMP pathway. This may contribute to the sinus tachycardia in pathological conditions associated with an increase in myocardial production of NO.

Key Words: nitric oxide • nitrovasodilator • heart rate • hyperpolarization-activated inward current •

sinoatrial node

Introduction

Top
Abstract
Introduction
Materials and Methods
Results
Discussion
References

Although the role of NO in modulating myocardial contractility has been extensively investigated, the effects of NO on heart rate have received comparatively little attention.[1] [2] It is well known that systemic administration of NO donors (eg, SNP) is associated with an increase in heart rate, and this is thought to be due to a neurally mediated reflex response to the fall in arterial blood pressure.[3] However, SNP can also increase heart rate in heart transplant recipients[4] before sympathetic reinnervation can occur,[5] suggesting that NO donors might stimulate SAN activity independent of the arterial baroreflex. The evidence supporting this hypothesis is, however, inconclusive. In isolated right atria, low concentrations of the NO donor SIN-1 had no effect on beating rate, whereas very high concentrations had a negative chronotropic effect.[6] Conversely, in a study aimed to assess the role of NO in modulating arrhythmias in isolated perfused hearts, Pabla and Curtis[7] noted an increase in beating rate in response to a low concentration of SNP and a negative chronotropic effect after blockade of the NO synthase with N^G-nitro-L-arginine methyl ester. This suggests that NO might independently stimulate pacemaker activity.

To test this hypothesis, we investigated whether exogenous NO could affect the spontaneous beating rate of an isolated guinea pig SAN/atrial preparation. We found that SNP and SIN-1 caused a biphasic, concentration-dependent, chronotropic response. The increase in beating rate was prevented by guanylyl cyclase inhibitors and could be mimicked by 8-Br-cGMP. Furthermore, this positive chronotropic effect was not affected by $I_{Ca\text{-}L}$ antagonism but was abolished by blockers of I_f. Finally, in rabbit isolated SAN cells, we showed a marked Cs^+-sensitive increase in I_f with both SNP and SIN-1. When taken together, these results indicate that the increase in rate with NO donors is due to stimulation of I_f via the NO-cGMP pathway.

Materials and Methods

Top
Abstract
Introduction
Materials and Methods
Results
Discussion
References

Experiments were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health) and the *Animals (Scientific Procedures) Act 1986* (UK).

Guinea Pig SAN/Atrial Preparation

Guinea pigs (400 to 450 g) of either sex were killed by cervical dislocation and exsanguinated. The heart was rapidly removed and placed in a dissecting dish with Tyrode's solution aerated with 95% O_2/5% CO_2 at 35°C to 37°C. Heparinized Tyrode's solution (1000 U/mL) was immediately perfused through the aorta, and the ventricles were carefully dissected and removed. Sutures (Ethicon 6/0 silk) were placed at the lateral edges of the two atria. The preparation was then transferred to a preheated (37±0.1°C), continuously oxygenated, water-jacketed bath containing 60 mL of Tyrode's solution. The atria were mounted vertically with the suture in the right atrium attached to a stainless steel hook, and the left atrium was attached to an isometric force transducer (HSE F30), which was connected to a laboratory-built amplifier. Data were acquired on a Power Macintosh 8500 computer using a Biopac MP100 data acquisition system and AcqKnowledge 3.5 software. Beating rate was triggered from contraction, and the signals were

displayed in real time. Data were stored on an optical disk for off-line analysis.

Solution and Drugs

The Tyrode's solution contained (mmol/L) NaCl 120, KCl 4, $MgCl_2$ 2, $NaHCO_3$ 25, $CaCl_2$ 1.8, NaH_2PO_4 0.1, and glucose 11. The solution was aerated with 95% O_2/5% CO_2 (pH 7.4), and its temperature was continuously monitored (Digitron 1408-K gauge) and kept at 37±0.1°C.

Two different NO donors, SNP (Sigma) and SIN-1 (Sigma Chemical Co),[8] were used. In addition, SNAP (Affiniti Ltd) was tested as an NO donor with *S*-nitrosylating properties.[9] CsCl (2 mmol/L, Sigma) and ZD7288 (1 µmol/L, Zeneca Pharmaceuticals) were used as selective blockers of I_f,[10] [11] [12] and NIF (0.2 µmol/L, Sigma) was used to antagonize $I_{Ca\text{-}L}$.[13] NIF (0.2 µmol/L) was used, because in a preliminary set of experiments, this concentration was the highest that elicited a stable bradycardia without arresting the preparation. SOD (Sigma), an enzyme known to enhance NO-dependent effects through scavenging the superoxide anion,[14] inhibitors of guanylyl cyclase LY83583[15] (Calbiochem) and ODQ[16] [17] (Tocris Cookson UK), and the membrane-permeable cGMP analogue 8-Br-cGMP[18] (Sigma) were used to evaluate the mechanism of the chronotropic effect of NO donors.

CsCl, ZD7288, and NIF were added from stock solutions of 1 mol/L, 1 mmol/L, and 0.1 mmol/L, respectively. Solutions of SNP or SNAP (in water of pH 7.4) and SIN-1 (in water of pH 5.4 to 5.8) were prepared immediately before application.[8] [9] All water used was of reagent grade from an Elga water purification system. Exchange of the solution during experiments (see "Protocols") was achieved from a jacketed reservoir kept at 37°C.

Protocols

Before starting each protocol, we kept the mounted atria in Tyrode's solution for 120 to 200 minutes (the medium was changed every 20 minutes), until their beating rate stabilized (within 5 bpm for 40 minutes). Since SNP, SIN-1,[8] 8-Br-cGMP, and NIF are very light-sensitive, all experiments were carried out in a darkened room.

Chronotropic Response to Incremental Concentrations of NO Donors

SNP (n=6) or SIN-1 (n=8) was applied cumulatively to the tissue bath in half-logarithmic increments (the next dose added after a stable response to the previous concentration was reached) to achieve a range of concentrations from $5x10^{-8}$ to 10^{-2} mol/L for SNP and from $5x10^{-8}$ to 10^{-3} mol/L for SIN-1. The concentration-response relation to SIN-1 was also determined (n=6) in the presence of SOD (50 to 100 U/mL) to minimize the possible role of superoxide (an agent generated in addition to NO during SIN-1 breakdown) or peroxynitrite (a product of NO and superoxide)[9] [19] in eliciting the chronotropic effect.

It is known that under physiological conditions NO can react with thiol groups in proteins to form *S*-nitrosothiols, which may serve as biologically active intermediates of NO.[20] Furthermore, *S*-nitrosylation (NO^+ transfer) can account for both cGMP-dependent[20] [21] and cGMP-independent[19] effects of NO. For these reasons, we also tested the chronotropic effect of increasing concentrations of the *S*-nitrosothiol SNAP[9] (n=7 plus n=3 control preparations for the effect of the carrier, *N*-acetyl-D,L-penicillamine; concentration range, from $5x10^{-8}$ to 10^{-3} mol/L).

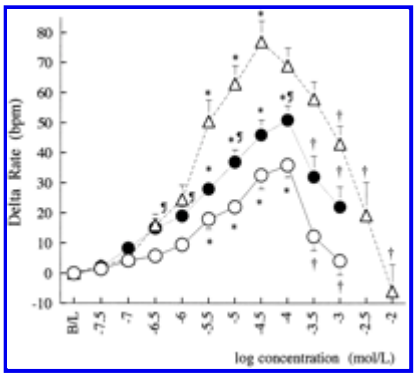
Role of cGMP in the Positive Chronotropic Response to Exogenous NO

Modulatory effects of NO donors on membrane channels can occur both via indirect (cGMP-dependent) and direct (redox-modulation) mechanisms.[19] [22] We investigated the role played by the cGMP-dependent pathway in the positive chronotropic effect of NO donors by evaluating (1) the chronotropic effect of increasing concentrations of a membrane-permeable analogue of cGMP, 8-Br-cGMP (10^{-6} to 10^{-3} mol/L,

n=6), and (2) the concentration-response relation to SIN-1 in the presence of 8-Br-cGMP (1 mmol/L, 20-minute preincubation, n=5) or in the presence of a guanylyl cyclase inhibitor, LY83583 (5µmol/L, 40-minute preincubation, n=6)[1] [15] [18] or ODQ (10 µmol/L, 40-minute preincubation, n=6).[16] [17]

Chronotropic Effect of SNP in the Presence of NIF

Each experiment was preceded by a control response to SNP (10 µmol/L, the concentration causing submaximal positive chronotropic effect; see Fig 1) and a washout. Subsequently, NIF (0.2 µmol/L, n=10) was added, and when a stable beating rate was reached, the same dose of SNP was reapplied. The time course of the experiment was as follows: SNP (10 minutes)→washout (20 minutes)→NIF (20 minutes)→SNP (10 minutes).



View larger version (23K):
[in this window]
[in a new window]

Figure 1. Concentration-dependent effect of NO donors on the spontaneous beating rate of guinea pig SAN/atrial preparations. Graph shows the mean data±SEM from separate SAN/atria preparations treated with SNP (n=6, △), SIN-1 (n=8, ○), or SIN-1 in the presence of SOD (50 to 100 U/mL, n=6, ●). SNP or SIN-1 was applied cumulatively in half-logarithmic increments (the next dose added after a stable response to the previous concentration was reached). B/L indicates baseline beating rate after stabilization. Note that both NO donors had a concentration-dependent biphasic effect on the beating rate, with a positive chronotropic response with lower concentrations and a decrease in rate at higher concentrations of the donors. The positive chronotropic effect of SIN-1 was significantly enhanced in the presence of SOD. *P<.05 vs B/L. †P<.05 vs the peak positive chronotropic response to a given NO donor. ¶P<.05 vs the response to SIN-1 without SOD.

Chronotropic Effect of SNP in the Presence of I_f Blockers

Experiments were preceded by a control response to SNP (10 µmol/L) and a washout. An I_f antagonist, either 2 mmol/L CsCl (n=10) or 1 µmol/L ZD7288 (n=8), was then added, and when a stable beating rate was reached, the same dose of SNP was reapplied. The time course of the experiment was as follows: SNP (10 minutes)→washout (20 minutes)→Cs^+ (15 minutes) or ZD7288 (45 minutes)→SNP (10 minutes).

Chronotropic Response to SIN-1 in the Presence of NIF or I_f Blockade

To evaluate whether some nonspecific properties of SNP[8] might affect the chronotropic response during I_f or $I_{Ca\text{-}L}$ blockade, the effect of 50 µmol/L SIN-1 (concentration causing submaximal effect; see Fig 1) on the beating rate was tested before and after treatment with NIF (0.2 µmol/L) or CsCl (2 mmol/L) as described above (n=8 in each series).

Isolated Rabbit SAN Cells

Cell Isolation and Solutions

Pacemaker cells were isolated from the SAN of New Zea-land White rabbits (700 to 900 g) killed by cervical dislocation. The isolation protocol and composition of external solution have been described in detail previously.[23] In brief, thin strips of SAN tissue (≈0.5x3 mm) were placed in Ca^{2+}-free Tyrode's solution for 5 minutes and subsequently incubated at 37°C for 30 to 40 minutes in the presence of collagenase (Sigma, 230 U/mL) and elastase (Sigma, 15 U/mL). After the strips were maintained in Krebs' buffer at 4°C for at least 1 hour, single cells were released from the tissue by glass pipette suction.

The whole-cell patch-clamp mode (amphotericin-permeabilized patches; internal solution containing

[mmol/L] KCl 140, HEPES 5, EGTA 1, and $MgSO_4$ 1.8, titrated to pH 7.4 with KOH, and amphotericin, 200 µg/mL) was used for electrical recordings from single SAN cells.

A temperature of 36±0.5°C was maintained throughout each experiment. For details regarding recording methods and data acquisition, see Reference 23[23] .

Protocols

In 21 cells, after successful seal formation and amphotericin permeabilization, a two-pulse voltage-clamp protocol was used to test for I_f from the holding potential of -40 to -70 mV (1 second) and then from -40 to -100 mV (1 second).

Effect of SNP on the Amplitude of I_f (n=11)

After a control recording, the solution was changed for the one containing 5 µmol/L SNP (prepared immediately before application), and subsequent recordings were made at 3, 5, and 10 minutes. Washout of SNP was attempted in six cells.

Effect of Cs^+ on I_f in the Presence of SNP (n=6)

The same two-pulse protocol was used (see above) to evaluate whether CsCl (2 mmol/L) inhibits the effect of SNP (5 µmol/L) on I_f. The time course of recordings was as follows: control→SNP (5 and 10 minutes after application)→SNP plus Cs^+ (5 and 10 minutes)→SNP only (5 and 10 minutes).

Control Experiments With SIN-1 (n=4)

The I_f protocol (as above) was used to test whether SIN-1 modulates I_f in a similar manner to that of SNP. In addition, in the same cells we evaluated the effect of SIN-1 on $I_{Ca\text{-}L}$. In all experiments, exposure to SNP or SIN-1 was performed in a darkened room.

Statistical Analysis

Data are presented as mean±SEM. For experiments on SAN/atrial preparations, one-way repeated measures ANOVA followed by Scheffé's post hoc test was used to evaluate the effect of increasing NO donor or 8-Br-cGMP concentrations on beating rate and to assess the effect of antagonists of pacemaker currents within the same group of experiments. One-way factorial ANOVA (followed by Scheffé's post hoc test) was used to compare the chronotropic effect of SIN-1 alone versus SIN-1 in the presence of SOD and the effect NO donors after the application of NIF versus I_f blockers. Student's *t* test was used to compare changes in the magnitude of I_f during exposure to SNP in isolated pacemaker cells and to evaluate the effect of Cs^+. Statistical significance was accepted at $P<.05$.

Results

Immediately after the SAN/atrial preparations were placed in the experimental chamber, the mean spontaneous beating rate was 254±3 bpm. During the period of stabilization (120 to 200 minutes), the beating rate decreased in an exponential fashion until it reached a stable value, which averaged 179±3 bpm (n=97). Six SAN/atrial preparations were discarded, since their beating rates did not remain stable.

Chronotropic Response to NO Donors

Fig 1 shows the chronotropic effect of increasing concentrations of SNP and SIN-1 on spontaneously beating SAN/atria. SNP caused a progressive increase in beating rate, which became significantly different

from baseline at concentrations from 5 to 50 µmol/L. The peak positive chronotropic response to SNP was reached at 50 µmol/L (rate increase of 77±7 bpm, *P*<.05). Further increments in SNP concentration resulted in a stepwise decrease in the beating rate. At the highest concentration of SNP used (10 mmol/L), the beating rate was lowered by 83±9 bpm (*P*<.05) compared with the average maximal positive chronotropic effect of this agent (Fig 1).

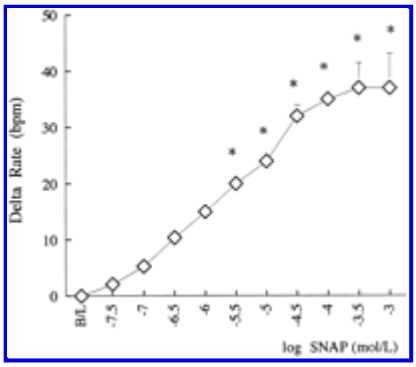
The concentration-response curve to SIN-1 was similar to that for SNP (Fig 1). However, the peak increase in the beating rate with SIN-1 (+36±4 bpm, *P*<.05) was significantly lower than that with SNP and occurred at 100 µmol/L. The highest concentration of SIN-1 (1 mmol/L) caused a decrease in spontaneous rate by 32±5 bpm compared with the peak rate achieved in response to this drug.

In the presence of SOD (Fig 1), the positive chronotropic effect of SIN-1 was significantly enhanced, with the peak increase in beating rate averaging 51±5 bpm (*P*<.05 versus the effect of SIN-1 alone).

In summary, both SNP and SIN-1 caused a biphasic concentration-dependent chronotropic response, with a gradual increase in beating rate for low concentrations and a decrease in beating rate for high concentrations of either NO donor. The response to SIN-1 was enhanced in the presence of SOD.

Chronotropic Response to SNAP

Incremental concentrations of SNAP, an *S*-nitrosylating compound and NO donor,[9] caused a progressive increase in beating rate, which became statistically significant for concentrations of ≥5 µmol/L and peaked at 0.5 and 1 mmol/L (increase of 37±5 and 37±6 bpm, *P*<.05, Fig 2). Conversely, *N*-acetyl-D,L-penicillamine, used in the same range of concentrations as SNAP, had no effect on the beating rate. At concentrations of 0.5 and 1 mmol/L, the positive chronotropic effect of SNAP was often preceded by a short-lived (1- to 2-minute) decrease in the beating rate.



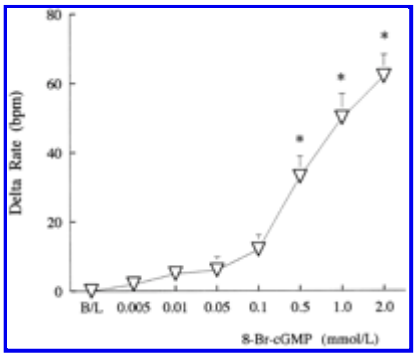
View larger version (14K):
[in this window]
[in a new window]

Figure 2. Concentration-dependent effect (mean±SEM) of the *S*-nitrosothiol SNAP on the spontaneous beating rate of guinea pig SAN/atrial preparations. SNAP (n=7) was applied cumulatively in half-logarithmic increments (the next dose added after a stable response to the previous concentration was reached). B/L indicates baseline beating rate after stabilization. Note a concentration-dependent increase of the beating rate with lower concentrations but the lack of an overall decrease in rate at higher concentrations of SNAP. **P*<.05 vs B/L.

Role of cGMP-Dependent Pathway

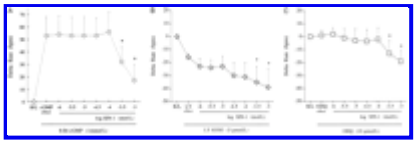
Application of increasing concentrations of 8-Br-cGMP resulted in an progressive increase in beating rate (Fig 3). The peak effect was observed at the highest concentration of 8-Br-cGMP (increase of 62±6 bpm, *P*<.05). In the presence of 8-Br-cGMP, the positive chronotropic effect of low concentrations of SIN-1 (≤0.1 mmol/L) was abolished while the decrease in rate in response to higher concentrations was still present (Fig 4A). LY83583[15] caused a nonsignificant decrease in the spontaneous rate (-8.6%) and prevented the positive (but not the negative) chronotropic effect of SIN-1 (Fig 4B). However, LY83583, in addition to inhibiting the guanylyl cyclase,[15] appears to have other biological actions that can affect NO-dependent

pathway(s), eg, generation of oxygen-derived free radicals[24] and direct inactivation of NO.[15] [25] For that reason, we also evaluated the concentration-response relation to SIN-1 in the presence of ODQ, a novel specific inhibitor of guanylyl cyclase.[16] [17] In the presence of ODQ, low concentrations of SIN-1 (≤0.1 mmol/L) did not alter the beating rate, whereas higher concentrations decreased it (Fig 4C).



View larger version (13K):
[in this window]
[in a new window]

Figure 3. Chronotropic effect of 8-Br-cGMP. Concentration-dependent chronotropic effect (mean±SEM) of 8-Br-cGMP in guinea pig SAN/atrial preparations (n=6; the next dose added after a stable response to the previous concentration was reached). B/L indicates baseline beating rate after stabilization. Note a concentration-dependent increase of the beating rate that mimics the positive chronotropic effect of NO donors shown in Fig 1. *P<.05 vs B/L.



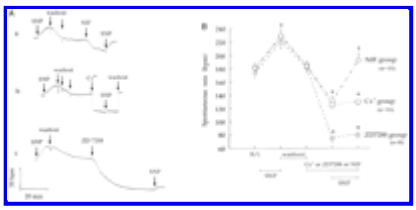
View larger version (12K):
[in this window]
[in a new window]

Figure 4. Role of endogenous cGMP in the positive chronotropic response to NO donors. A, Chronotropic effect of incremental concentrations of SIN-1 in the presence of 8-Br-cGMP (1 mmol/L). Note that SIN-1 had no additional positive chronotropic effect after the beating rate increased in response to 8-Br-cGMP. *P<.05 vs the effect of 8-Br-cGMP alone. B, Concentration-response relation to SIN-1 in the presence of LY83583. Note that (1) LY83583 decreases the beating rate and (2) high concentrations of SIN-1 have a further negative chronotropic effect in the presence of this agent. *P<.05 vs baseline beating rate after stabilization (B/L). C, Chronotropic effect of increasing concentrations of SIN-1 in the presence of a novel specific guanylyl cyclase inhibitor, ODQ. Note that the increase in beating rate with low concentrations of the NO donor (seen in Fig 1) is completely prevented by ODQ but that the negative chronotropic effect of high concentrations of SIN-1 is still present.

In summary, the positive chronotropic response to NO donors (1) was prevented by LY83583 or ODQ and (2) was mimicked by a membrane-permeable analogue of cGMP with no additional effect of the NO donor in its presence.

Effect of Antagonizing $I_{Ca\text{-}L}$ on the Positive Chronotropic Response to SNP

Fig 5A (trace a) shows representative raw data of the effect of SNP (10 µmol/L) on the beating rate before and after $I_{Ca\text{-}L}$ was antagonized with NIF (0.2 µmol/L). The control response to SNP resulted in an average increase in beating rate of 49±9 bpm (P<.05), which was fully reversed after washout of SNP. NIF decreased the beating rate from 184±7 to 131±8 bpm (-29%, P<.05). When SNP was reapplied in the presence of NIF, it still caused an increase in the beating rate of 61±14 bpm (P<.05, Fig 5A, trace a, and Fig 5B).



View larger version (12K):
[in this window]
[in a new window]

Figure 5. A, Representative raw data traces showing the effect of SNP (0.01 mmol/L) on the beating rate of SAN/atrial preparations when I_{Ca-L} was antagonized by 0.2 µmol/L NIF (trace a) and when I_f was blocked by 2 mmol/L Cs^+ (trace b) or 1 µmol/L ZD7288 (trace c). Experiments were conducted in a darkened room and were preceded by a control response to SNP. Washouts are denoted by arrow(s) below the "washout" label. B, Mean data for 10 experiments in which NIF was used to antagonize I_{Ca-L} (○), 10 experiments in which Cs^+ was used to block I_f (□), and 8 experiments in which ZD7288 was used to block I_f (△). Note that the positive chronotropic response to SNP was almost completely abolished in the presence of I_f blockers but was intact in the presence of NIF. *P<.05 vs baseline beating rate after stabilization (B/L) and postwashout value (for each of the three groups separately). †P<.05 vs the response to SNP when I_f had been blocked by Cs^+ or ZD7288.

In summary, the positive chronotropic response to the NO donor SNP was maintained when the L-type Ca^{2+} current was antagonized by NIF.

Effect of Blocking I_f on the Positive Chronotropic Response to SNP

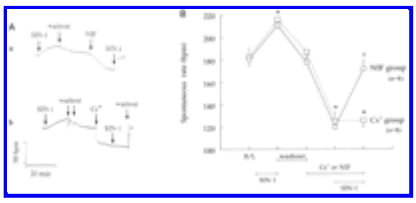
We tested whether applying 2 mmol/L CsCl or 1 µmol/L ZD7288 to block I_f would attenuate the positive chronotropic response to SNP. Fig 5A (trace b and trace c) shows examples of raw data from these experiments (mean values are shown in Fig 5B). SNP (10 µmol/L) caused a comparable increase in beating rate in both groups (by 45±7 bpm in the group in which Cs^+ was subsequently applied and by 46±8 bpm in the ZD7288 group, P<.05 for either group), which was fully reversed after washout. Cs^+ (2 mmol/L) and ZD7288 (1 µmol/L) decreased the spontaneous rate by 60±3 bpm (-32%, P<.05) and 106±7 bpm (-58%, P<.05), respectively. When applied in the presence of either I_f blocker, SNP no longer had a significant positive chronotropic effect (5±2 bpm, P=NS).

In summary, the positive chronotropic response to SNP was virtually abolished in the presence of I_f blockade with either Cs^+ or ZD7288.

Effect of NIF Versus Cs^+ on the Positive Chronotropic Response to SIN-1

To test whether the chronotropic effect of SNP could be attributed to some nonspecific properties of this agent,[8] we repeated our experiments (n=8 for NIF and n=8 for Cs^+) using the NO donor SIN-1 (50 µmol/L).

In Fig 6A, two original rate traces are shown, one from the NIF group (trace a) and one from the Cs^+ group (trace b); data for all experiments are summarized in Fig 6B. SIN-1 increased the beating rate by 30±7 bpm in the NIF group and 33±5 bpm in the Cs^+ group (P<.05 for either group), and this was fully reversed after washout. Application of NIF (0.2 µmol/L) or Cs^+ (2 mmol/L) caused a comparable significant decrease in rate by 58±10 bpm (-32%) and 62±7 bpm (-33%), respectively (P=NS for differences between the two groups). In the presence of NIF, SIN-1 increased the beating rate by 51±12bpm (P<.05). After the application of Cs^+, however, the positive chronotropic effect of SIN-1 was abolished (+1±1 bpm, P=NS).



View larger version (11K):
[in this window]
[in a new window]

Figure 6. A, Representative raw data traces showing the effect of SIN-1 (0.05 mmol/L) on the beating rate of SAN/atrial preparations when $I_{Ca\text{-}L}$ was antagonized with 0.2 µmol/L NIF (trace a) and when I_f was blocked by 2 mmol/L Cs^+ (trace b). Each experiment was preceded by a control response to SIN-1. Washouts are denoted by arrow(s) below the "washout" label. B, Mean data for eight experiments in which NIF was used to antagonize $I_{Ca\text{-}L}$ (○) and eight experiments in which Cs^+ was used to block I_f (□). Note that positive chronotropic response to SIN-1 was virtually abolished in the presence of Cs^+ but was intact in the presence of NIF. *P<.05 vs baseline beating rate after stabilization (B/L) and postwashout value (for each of the two groups separately). †P<.05 vs the response to SIN-1 when I_f had been antagonized with Cs^+.

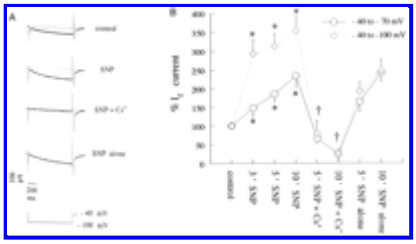
In summary, the increase in beating rate in response to SIN-1 was maintained in the presence of NIF but was completely prevented by blocking I_f with Cs^+.

Effect of SNP and SIN-1 on I_f in Single SAN Pacemaker Cells

Consistent with previous reports,[26] the control amplitude of I_f varied in different cells from -5 to -113 pA for the first hyperpolarizing voltage-clamp pulse (-40 to -70 mV) and from -10 to -242 pA for the second pulse (-40 to -100 mV).

Effect of SNP on the Amplitude of I_f

After exposure to SNP, the amplitude of I_f activated by the first pulse increased in all but one cell, whereas all cells showed an increase in I_f in response to the second pulse (in one cell, the patch was lost before the recording at 10 minutes). With the first pulse, the average increase in I_f with SNP was 48±21% at 3 minutes, 85±20% at 5 minutes, and 134±19% at 10 minutes (P<.05, Fig 7B). The corresponding values with the second pulse were 193±38%, 213±33%, and 254±38% (P<.05, Fig 7B).



View larger version (15K):
[in this window]
[in a new window]

Figure 7. Effect of the NO donor SNP on I_f in isolated rabbit SAN cells. Amphotericin-permeabilized patch technique was used. Cells were voltage-clamped at -40 mV, and the magnitude of I_f in response to 1-second hyperpolarizations (pulses from -40 to -70 mV and from -40 to -100 mV) was measured. I_f was recorded before and at 3, 5, and 10 minutes after exposure to 5 µmol/L SNP in a darkened room (n=17 cells). In n=6 cells, additional recordings were obtained 5 and 10 minutes after the addition of 2 mmol/L CsCl and then 5 and 10 minutes after washout of Cs^+ (in the presence of SNP). A, from top to bottom, Representative chronological recordings of I_f (pulse from -40 to -100 mV) from the same cell in control, after 10 minutes of exposure to SNP, after 10 minutes of exposure to SNP and Cs^+, and 10 minutes after Cs^+ washout. B, Average magnitude of normalized I_f with the pulse from -40 to -70 mV (○) and from -40 to -100 mV (◇) before and 3, 5, and 10 minutes after exposure to SNP, 5 and 10 minutes after the addition of Cs^+, and 5 and 10 minutes after Cs^+ washout. Note that SNP increased the magnitude of I_f. This effect was time dependent and reversibly suppressed by Cs^+ (see text for details). *P<.05 vs the control amplitude of the current with each pulse. †P<.05 vs the amplitude of I_f after 10 minutes of exposure to SNP.

Washout of the NO donor was attempted in six cells: in three cells, a full reversal of the amplitude of I_f was observed; in two, the patch was lost after the solution was changed; and in one, the magnitude of the current was not back to the control value after 25 minutes.

Effect of Cs^+ on I_f in the Presence of SNP

The stimulation of I_f by 5 µmol/L SNP was suppressed 5 minutes after the application of 2 mmol/L CsCl in the presence of SNP (from 234±19% to 30±12% of the control value for the first pulse and from 354±38% to 78±36% of the control value for the second pulse, P<.05 for both pulses) (Fig 7A and Fig 7B). After 10 minutes of exposure to Cs^+ in the presence of SNP, I_f could not be elicited by the first pulse in 50% of the cells (average amplitude, 24±16% of the control value; P<.05; Fig 7B), whereas the mean amplitude of I_f with the second pulse was 27±8% of the control value (P<.05, Fig 7B). Ten minutes after Cs^+ washout, the amplitude of I_f was 242±24% of the control value during the first pulse and 247±29% of the control value during the second pulse.

Effect of SIN-1 on I_f and $I_{Ca\text{-}L}$

SIN-1 (n=4) consistently increased the amplitude of I_f in all studied cells (I_f amplitude averaged 278±115% of the control value at 3 minutes and 336±194% after 5 minutes at -100 mV), but it did not stimulate $I_{Ca\text{-}L}$ ($I_{Ca\text{-}L}$ amplitude was 97±10% of the control value at 3 minutes and 94±12% after 5 minutes of exposure to SIN-1).

In summary, in isolated SAN cells the amplitude of the pacemaker current, I_f, was increased by SNP or SIN-1 (5 µmol/L). This effect was markedly and reversibly suppressed by 2 mmol/L Cs^+. In contrast, the amplitude of $I_{Ca\text{-}L}$ was not increased.

Discussion

Top
Abstract
Introduction
Materials and Methods
Results
Discussion
References

The new findings from this study are as follows: (1) NO donors modulate mammalian heart rate in a concentration-dependent biphasic fashion, with a gradual increase in beating rate for low concentrations and a decrease in beating rate for high concentrations. (2) The positive chronotropic effect appears to be NO-mediated and cGMP dependent. (3) The increase in beating rate with NO donors is maintained in the presence of the L-type Ca^{2+} channel antagonist NIF, but it is virtually abolished after I_f blockade. (4) Direct recordings in rabbit isolated SAN cells showed a marked, reversible, and Cs^+-sensitive increase in I_f with SNP or SIN-1, whereas $I_{Ca\text{-}L}$ was not increased.

Chronotropic Effect of NO Donors

Previous studies of the effect of exogenous NO on the beating rate of mammalian heart in vitro produced inconsistent results. In the isolated rat right atrium, Kennedy et al[6] showed that concentrations of SIN-1 from 0.01 μmol/L to 0.3 mmol/L did not significantly affect the beating rate, whereas higher concentrations decreased it. Conversely, Pabla and Curtis[7] noted an increase in the beating rate of 20% in Langendorff-perfused rat hearts in response to 10 μmol/L SNP. Furthermore, in this preparation, pharmacological blockade of endogenous NO synthase was associated with a reduction in beating rate by 15%.[7] This indicates that endogenously released NO might exert a tonic positive chronotropic effect that can be mimicked by NO donors.

The greater magnitude of the positive chronotropic response to SNP compared with SIN-1 (Fig 1) may be consistent with the different mechanisms by which these donors release NO. SNP has been reported to generate NO intracellularly,[27] whereas SIN-1 releases NO in aqueous solution, and this is rapidly scavenged in oxygenated buffer.[8] Since NO^+ can serve as a biologically relevant intermediate of NO[21] and since the modulation of membrane channels by NO^+ can differ from that by the free radical NO,[19] it was important to evaluate the chronotropic effect of an NO^+-donating compound. Interestingly, we found that incremental concentrations of the *S*-nitrosothiol SNAP[9] can elicit a progressive increase in beating rate similar to that produced by the NO donors SNP or SIN-1.[8 9] However, unlike SNP or SIN-1, SNAP did not produce a persistent negative chronotropic effect in high concentrations (Fig 3). Thus, an increase in beating rate could be elicited by both an *S*-nitrosothiol and NO donors (at least in nanomolar to micromolar concentrations). This is consistent with data showing that *S*-nitrosothiols can serve as guanylyl cyclase–stimulating intermediates of NO and NO donors.[20 21]

Increase in Beating Rate Is Due to NO and Occurs via a cGMP-Dependent Mechanism

In many tissues, NO is known to exert its effects through the stimulation of guanylyl cyclase and the increase in cGMP.[1 2] Our findings provide evidence for the involvement of NO-cGMP pathways in the positive chronotropic effect of NO donors. In particular, the enhancement of the chronotropic response to SIN-1 in the presence of SOD (Fig 1) is consistent with the primary involvement of NO. Furthermore, we show that inhibition of endogenous guanylyl cyclase by LY83583 or ODQ prevents the increase in beating rate with SIN-1 (Fig 4B and 4C), whereas the membrane-permeable analogue of cGMP, 8-Br-cGMP, can mimic it (Fig 3). Finally, in the presence of 8-Br-cGMP, SIN-1 did not produce an additional positive chronotropic effect (Fig 4A). These data are consistent with the involvement of cGMP in eliciting the positive chronotropic response to NO donors.

Functional Evidence That I_f Mediates the Positive Chronotropic Response to NO

Positive Chronotropic Effect of NO Donors Is Abolished in the Presence of Cs^+ or ZD7288

I_f is a highly modulated current that plays an important role in maintaining pacemaker activity[10] [26] [28] and in mediating the chronotropic response to autonomic agonists.[26] [28] Consistent with other reports,[11] [29] we found that the reduction in spontaneous beating rate of SAN/atrial preparations was greater with ZD7288 (1 μmol/L) than with Cs^+ (2 mmol/L). Both of these blockers of I_f, however, were equally effective in preventing the positive chronotropic effect of the NO donor SNP (Fig 5). Likewise, the increase in beating rate in response to SIN-1 could not be elicited in the presence of Cs^+ (Fig 6).

The ability of I_f blockers to prevent the increase in the beating rates of SAN/atrial preparations in response to SNP and SIN-1 indicates that (1) the positive chronotropic effect of NO results from the modulation of I_f in cardiac pacemaker cells and (2) the mechanism underlying the positive chronotropic effect is common for both NO donors.

Positive Chronotropic Effect of NO Donors Is Intact in the Presence of NIF

I_{Ca-L} is essential for myocardial contraction and for pacemaking in the SAN.[26] In isolated pacemaker cells, this current is selectively blocked by NIF.[13] The lack of attenuation of the NO-induced increase in the beating rate after pretreatment with NIF (Figs 5 and 6) indicates that stimulation of I_{Ca-L} in the cardiac pacemaker cells is unlikely to play a major role in the positive chronotropic effect of exogenous NO.

Stimulation of I_f in Isolated Pacemaker Cells

In isolated pacemaker cells, SNP or SIN-1 (5 μmol/L) caused a time-dependent increase in I_f (Fig 7), which was suppressed by 2 mmol/L CsCl. This is consistent with a recent finding by Janigro et al,[30] who showed that the I_f-like current in endothelial cells of the blood-brain barrier was markedly increased by low concentrations of SNP (from 1 to 10 μmol/L) and by 1 μmol/L SIN-1. Our results indicate that the increase in the beating rate in response to exogenous NO is primarily mediated by stimulation of I_f and not I_{Ca-L}. This is in keeping with data from other groups showing that NO donors have no effect on basal I_{Ca-L} in isolated cells from the SAN[18] or atrioventricular node.[31]

We have shown that the positive chronotropic effect of NO donors can be mimicked by increasing concentrations of a membrane-permeable analogue of cGMP. Interestingly, DiFrancesco[28] has demonstrated that I_f can be stimulated by cGMP in a concentration-dependent fashion. These data are consistent with our hypothesis that activation of the NO-cGMP-I_f pathway is responsible for the chronotropic effect of NO donors.

Our data provide evidence for stimulation of I_f by exogenous NO in rabbit isolated pacemaker cells; the work by Han et al[18] showed that NO participates in the cholinergic inhibition of isoproterenol-stimulated I_{Ca-L} in the same preparation. This suggests that NO can play an important role in promoting both the positive chronotropic effects[32] and the heart rate deceleration associated with vagal reflexes.[33] [34]

Clinical Implications

NO donors are widely used in cardiology, and our results suggest that they can have a biphasic concentration-dependent effect on pacemaking in the heart. Several in vivo observations support our findings on the SAN/atrial preparation. For instance, low doses of molsidomine (the prodrug of SIN-1) can increase heart rate without significantly affecting arterial blood pressure.[35] Likewise, the intracoronary injection of a low dose of SNP has been shown to increase the rate of canine hearts in situ in the absence of changes in arterial pressure.[36] Conversely, a slight reduction in heart rate was observed when 50-fold-higher

doses of SNP were used in a similar experiment in humans.[37]

From our results using exogenous NO, it could be extrapolated that stimulation of I_f by endogenous NO might play a part in the sinus tachycardia that accompanies pathological conditions associated with an increase in both sympathetic activity and myocardial production of NO (eg, septic shock and heart failure).[38] [39] Moreover, I_f has been recently found in ventricular myocytes from diseased human hearts,[40] suggesting that our findings might have wider implications for the role of NO in the performance of the failing heart.

Selected Abbreviations and Acronyms

8-Br-cGMP	=	8-bromoguanosine 3':5'-cyclic monophosphate
$I_{Ca\text{-}L}$	=	L-type Ca^{2+} current
I_f	=	hyperpolarization-activated inward current
LY83583	=	6-anilino-5,8-quinolinedione
NIF	=	nifedipine
NO	=	nitric oxide (and its congeners)
ODQ	=	1*H*-(1,2,4)oxadiazolo(4,3-*a*)quinoxalin-1-one
SAN	=	sinoatrial node
SIN-1	=	3-morpholinosydnonimine
SNAP	=	*S*-nitroso-*N*-acetyl-D,L-penicillamine
SNP	=	sodium nitroprusside
SOD	=	superoxide dismutase
ZD7288	=	4-(*N*-ethyl-*N*-phenylamino)-1,2-dimethyl-6-(methylamino)pyrimidinium chloride

Acknowledgments

We gratefully acknowledge the support of the British Heart Foundation and the Garfield Weston Trust. Dr Musialek is a recipient of an Overseas Research Award (UK) and is supported by the Wellcome Trust and by Hoechst Pharmaceuticals, Germany. Dr Lei is supported by the Medical Research Council, UK.

Received November 22, 1996; accepted April 15, 1997.

References

- Top
- Abstract
- Introduction
- Materials and Methods
- Results
- Discussion
- References

1. Kelly RA, Balligand J-L, Smith TW. Nitric oxide and cardiac function. *Circ Res.* 1996;79:363-380.[Full Text]
2. Shah A. Paracrine modulation of heart cell function by endothelial cells. *Cardiovasc Res.* 1996;31:847-867.[Medline]
3. Chen RY, Fan FC, Schuessler GB, Chien S. Baroreflex control of heart rate in humans during nitroprusside-induced hypotension. *Am J Physiol.* 1982;243:R18-R24.[Medline]

4. Levine TB, Olivari MT, Cohn JN. Effects of orthotopic heart transplantation on sympathetic control mechanisms in congestive heart failure. *Am J Cardiol.* 1986;58:1035-1040.[Medline]
5. De Marco T, Dae M, Yuen-Green MSF, Kumar S, Sudhir K, Keith F, Amidon T, Rifkin C, Klinski C, Lau D, Botvinick EH, Chatterjee K. Iodine-123 metaiodobenzylguanidine scintigraphic assessment of the transplanted human heart: evidence for late reinnervation. *J Am Coll Cardiol.* 1995;25:927-931.[Medline]
6. Kennedy RH, Hicks KK, Brian JE, Seifen E. Nitric oxide has no chronotropic effect in right atria isolated from rat heart. *Eur J Pharmacol.* 1994;225:149-156.
7. Pabla R, Curtis MJ. Effects of NO modulation on cardiac arrhythmias in the rat isolated heart. *Circ Res.* 1995;77:984-992.[Abstract/Full Text]
8. Feelisch M. The biochemical pathways of nitric oxide formation from nitrovasodilators: appropriate choice of exogenous NO donors and aspects of preparation and handling of aqueous NO solutions. *J Cardiovasc Pharmacol.* 1991;17(suppl 3):S25-S33.
9. Feelisch M, Stamler JS. Donors of nitrogen oxides. In: Feelisch M, Stamler JS, eds. *Methods in Nitric Oxide Research.* New York, NY: John Wiley & Sons Inc; 1996:71-115.
10. Denyer JC, Brown HF. Pacemaking in rabbit isolated sino-atrial node cells during Cs^+ block of the hyperpolarization-activated current I_f. *J Physiol (Lond).* 1990;429:401-409.[Abstract]
11. Leitch SP, Sears CE, Brown HF, Paterson DJ. Effects of high potassium and the bradycardic agents ZD7288 and cesium on heart rate of rabbits and guinea pigs. *J Cardiovasc Pharmacol.* 1995;25:300-306.[Medline]
12. BoSmith RE, Briggs I, Sturgess NC. Inhibitory actions of ZENECA ZD7288 on whole-cell hyperpolarization activated inward current (I_f) in guinea-pig dissociated sinoatrial node cells. *Br J Pharmacol.* 1993;110:343-349.[Abstract]
13. Hagiwara N, Irisawa H, Kameyama M. Contribution of two types of calcium currents to the pacemaker potentials of rabbit sino-atrial node cells. *J Physiol (Lond).* 1988;395:233-253.[Abstract]
14. Gryglewski RJ, Palmer RMJ, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature.* 1986;320:454-456.[Medline]
15. Mülsch A, Busse R, Liebau S, Förstermann U. LY83583 interferes with the release of endothelium-derived relaxing factor and inhibits soluble guanylate cyclase. *J Pharmacol Exp Ther.* 1988;247:283-288.[Abstract]
16. Cellek S, Kasakov L, Moncada S. Inhibition of nitrergic relaxations by a selective inhibitor of the soluble guanylate cyclase. *Br J Pharmacol.* 1996;118:137-140.[Abstract]
17. Brunner F, Schmidt K, Nielsen EB, Mayer B. Novel guanylyl cyclase inhibitor potently inhibits cyclic GMP accumulation in endothelial cells and relaxation of bovine pulmonary artery. *J Pharmacol Exp Ther.* 1996;277:48-53.[Abstract]
18. Han X, Shimoni Y, Giles WR. A cellular mechanism for nitric oxide-mediated cholinergic control of mammalian heart rate. *J Gen Physiol.* 1995;106:45-65.[Abstract]
19. Campbell DL, Stamler JS, Strauss HC. Redox modulation of L-type calcium channels in ferret ventricular myocytes. *J Gen Physiol.* 1996;108:277-293.[Abstract]
20. Stamler JS, Simon DI, Osborne JA, Mullins ME, Jaraki O, Michel T, Singel DJ, Loscalzo J. S-Nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds. *Proc Natl Acad Sci U S A.* 1992;89:444-448.[Abstract]
21. Ignarro LJ, Lippton H, Edwards JC, Baricos WH, Hyman AL, Kadowitz PJ, Gruetter CA. Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: evidence for the involvement of S-nitrosothiols as active intermediates. *J Pharmacol Exp Ther.* 1981;218:739-749.[Medline]
22. Bolotina VM, Najibi S, Palacino JJ, Pagano PJ, Cohen RA. Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature.* 1994;368:850-853.[Medline]
23. Lei M, Brown HF. Two components of the delayed rectifier potassium current I_K in rabbit sino-atrial node cells. *Exp Physiol.* 1996;81:725-741.[Medline]
24. Kontos HA, Wei EP. Hydroxyl radical–dependent inactivation of guanylate cyclase in cerebral arterioles by methylene blue and by LY83583. *Stroke.* 1993;24:427-434.[Abstract]
25. Barbier AJ, Lefebvre RA. Effect of LY83583 on relaxation induced by non-adrenergic non-cholinergic nerve stimulation and exogenous nitric oxide in the rat gastric fundus. *Eur J*

Pharmacol. 1992;219:331-334.[Medline]
26. Irisawa H, Brown HF, Giles W. Cardiac pacemaking in the sinoatrial node. *Physiol Rev.* 1993;73:197-227.[Medline]
27. Fung HL, Kowaluk EA, Chung SJ, Jhun BH, Seth P. Nitric oxide generation from nitrovasodilators in coronary artery smooth muscle cells is mediated by multiple enzymes. In: Moncada S, Marletta MA, Hibbs JB Jr, Higgs EA, eds. *The Biology of Nitric Oxide: Physiological and Clinical Aspects*. London, UK: Portland Press; 1992;1:139-141.
28. DiFrancesco D. The onset and autonomic regulation of pacemaker activity: relevance of the f current. *Cardiovasc Res.* 1995;29:449-456.[Medline]
29. Cai Q, Lei M, Brown HF. Responses of guinea-pig SA node/atria to acetylcholine and adrenaline in the presence of blockers of I_f and $I_{K,ACh}$. *J Physiol (Lond).* 1995;483:21P. Abstract.
30. Janigro D, West GA, Nguyen T-S, Winn HR. Regulation of blood-brain barrier endothelial cells by nitric oxide. *Circ Res.* 1994;75:528-538.[Abstract]
31. Han X, Kobzik L, Balligand J-L, Kelly RA, Smith TW. Nitric oxide synthase (NOS3)–mediated cholinergic modulation of Ca^{2+} current in adult rabbit atrioventricular nodal cells. *Circ Res.* 1996;78:998-1008.[Abstract/Full Text]
32. Reid IA, Chou L. Role of nitric oxide in the renin and heart rate responses to ß-adrenergic stimulation. *Hypertension.* 1994;23(suppl I):I-49-I-53.
33. Sears CE, Paterson DJ. Role of nitric oxide in the rate and contraction responses to acetylcholine following adrenergic stimulation in the isolated frog heart. *J Physiol (Lond).* 1996;495:167P-168P. Abstract.
34. Conlon K, Collins T, Kidd C. Modulation of vagal actions on heart rate produced by inhibition of nitric oxide synthase in the anaesthetised ferret. *Exp Physiol.* 1996;81:547-550.[Medline]
35. Malcolm AD. Clinical and hemodynamic effects of the new dilator drug molsidomine. *Am Heart J.* 1985;109:674-677.[Medline]
36. Crystal GJ, Gurevicius J. Nitric oxide does not modulate myo-cardial contractility acutely in *in situ* canine hearts. *Am J Physiol.* 1996;270:H1568-H1576.[Medline]
37. Paulus WJ, Vantrimpont PJ, Shah AM. Acute effects of nitric oxide on left ventricular relaxation and diastolic distensibility in humans: assessment by bicoronary sodium nitroprusside infusion. *Circulation.* 1994;89:2070-2078.[Abstract]
38. Haywood GA, Tsao PS, von der Leyen HE, Mann MJ, Keeling PJ, Trindade PT, Lewis NP, Byrne CD, Rickenbacher PR, Bishopric NH, Cooke JP, McKenna WJ, Fowler MB. Expression of inducible nitric oxide synthase in human heart failure. *Circulation.* 1996;93:1087-1094.[Abstract/Full Text]
39. Ungureanu-Longrois D, Balligand J-L, Kelly RA, Smith TW. Myocardial contractile dysfunction in the systemic inflammatory response syndrome: role of a cytokine-inducible nitric oxide synthase in cardiac myocytes. *J Mol Cell Cardiol.* 1995;27:155-167.[Medline]
40. Cerbai E, Pino R, Porciatti F, Sani G, Toscano M, Maccherini M, Giunti G, Mugelli A. Characterization of the hyperpolarization-activated current, I_f, in ventricular myocytes from human failing heart. *Circulation.* 1997;95:568-571.[Abstract/Full Text]

This article has been cited by other articles:

- Narkiewicz, K., van de Borne, P. J.H., Hausberg, M., Cooley, R. L., Winniford, M. D., Davison, D. E., Somers, V. K. (1998). Cigarette Smoking Increases Sympathetic Outflow in Humans. *Circulation* 98: 528-534 [Abstract] [Full Text]
- Chandler, M. P., Rodenbaugh, D. W., DiCarlo, S. E. (1998). Arterial baroreflex resetting mediates postexercise reductions in arterial pressure and heart rate. *Am. J. Physiol.* 275: 1627H-1634 [Abstract] [Full Text]
- Sears, C. E., Choate, J. K., Paterson, D. J. (1999). NO-cGMP pathway accentuates the decrease in heart rate caused by cardiac vagal nerve stimulation. *J. Appl. Physiol.* 86: 510-516 [Abstract] [Full Text]

- Abstract of this Article
- Email this article to a friend
- Similar articles found in:
  Circulation Research Online
  PubMed
- PubMed Citation
- This Article has been cited by:
- Search Medline for articles by:
  Musialek, P. || Casadei, B.
- Alert me when:
  new articles cite this article
- Download to Citation Manager

- Slovut, D. P., Wenstrom, J. C., Moeckel, R. B., Salerno, C. T., Park, S. J., Osborn, J. W. (1999). Beat-to-beat modulation of heart rate is coupled to coronary perfusion pressure in the isolated heart. *J. Appl. Physiol.* 86: 694-700 [Abstract] [Full Text]
- Carmeliet, E. (1999). Cardiac Ionic Currents and Acute Ischemia: From Channels to Arrhythmias. *Physiol. Rev* 79: 917-1017 [Abstract] [Full Text]
- (1998). . *J. Appl. Physiol.* 84: 1596-1603 [Full Text]
- Hogan, N., Kardos, A., Paterson, D. J., Casadei, B. (1999). Effect of exogenous nitric oxide on baroreflex function in humans. *Am. J. Physiol.* 277: 221H-227 [Abstract] [Full Text]
- Hogan, N., Casadei, B., Paterson, D. J. (1999). Nitric oxide donors can increase heart rate independent of autonomic activation. *J. Appl. Physiol.* 87: 97-103 [Abstract] [Full Text]
- Janssen, B. J. A., Leenders, P. J. A., Smits, J. F. M. (2000). Short-term and long-term blood pressure and heart rate variability in the mouse. *Am. J. Physiol.* 278: 215R-225 [Abstract] [Full Text]
- Sener, A., Smith, F. G. (2001). Nitric oxide modulates arterial baroreflex control of heart rate in conscious lambs in an age-dependent manner. *Am. J. Physiol.* 280: 2255H-2263 [Abstract] [Full Text]
- Chesnais, J.-M., Fischmeister, R., Méry, P.-F. (1999). Positive and negative inotropic effects of NO donors in atrial and ventricular fibres of the frog heart. *J Physiol Lond* 518: 449-461 [Abstract] [Full Text]
- Chesnais, J.-M., Fischmeister, R., Méry, P.-F. (1999). Peroxynitrite is a positive inotropic agent in atrial and ventricular fibres of the frog heart. *J Physiol Lond* 521: 375-388 [Abstract] [Full Text]
- Müller-Strahl, G., Kottenberg, K., Zimmer, H.-G., Noack, E., Kojda, G. (2000). Inhibition of nitric oxide synthase augments the positive inotropic effect of nitric oxide donors in the rat heart. *J Physiol Lond* 522: 311-320 [Abstract] [Full Text]
- Martínez-Nieves, B., Dunbar, J. C. (1999). Vascular Dilatatory Responses to Sodium Nitroprusside (SNP) and {alpha}-Adrenergic Antagonism in Female and Male Normal and Diabetic Rats. *EXP BIOL MED* 222: 90-98 [Abstract] [Full Text]
- Brahmajothi, M. V., Campbell, D. L. (1999). Heterogeneous Basal Expression of Nitric Oxide Synthase and Superoxide Dismutase Isoforms in Mammalian Heart : Implications for Mechanisms Governing Indirect and Direct Nitric Oxide-Related Effects. *Circulation Research* 85: 575-587 [Abstract] [Full Text]
- Burger, H. R., Chandler, M. P., Rodenbaugh, D. W., DiCarlo, S. E. (1998). Dynamic exercise shifts the operating point and reduces the gain of the arterial baroreflex in rats. *Am. J. Physiol.* 275: 2043R-2048 [Abstract] [Full Text]
- Herring, N., Paterson, D. J. (2001). Nitric oxide-cGMP pathway facilitates acetylcholine release and bradycardia during vagal nerve stimulation in the guinea-pig in vitro. *J Physiol Lond* 535: 507-518 [Abstract] [Full Text]
- Choate, J. K., Danson, E. J. F., Morris, J. F., Paterson, D. J. (2001). Peripheral vagal control of heart rate is impaired in neuronal NOS knockout mice. *Am. J. Physiol.* 281: H2310-2317 [Abstract] [Full Text]
- Herring, N., Zaman, J. A. B., Paterson, D. J. (2001). Natriuretic peptides like NO facilitate cardiac vagal neurotransmission and bradycardia via a cGMP pathway. *Am. J. Physiol.* 281: H2318-2327 [Abstract] [Full Text]
- Chowdhary, S., Vaile, J. C., Fletcher, J., Ross, H. F., Coote, J. H., Townend, J. N. (2000). Nitric Oxide and Cardiac Autonomic Control in Humans. *Hypertension* 36: 264-269 [Abstract] [Full Text]