Nitric oxide donors can increase heart rate independent of autonomic activation

NIALL HOGAN,[1] BARBARA CASADEI,[2] AND DAVID J. PATERSON[1]
[1]*University Laboratory of Physiology, Oxford OX1 3PT; and* [2]*Department of Cardiovascular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, United Kingdom*

Hogan, Niall, Barbara Casadei, and David J. Paterson. Nitric oxide donors can increase heart rate independent of autonomic activation. *J. Appl. Physiol.* 87(1): 97–103, 1999.—Administration of nitric oxide (NO) donors in vivo is accompanied by a baroreflex-mediated increase in heart rate (HR). In vitro, however, NO donors can increase HR directly by stimulating a pathway that involves NO, cGMP, and the hyperpolarization-activated current (I_f). The aim of this study was to assess the functional significance of this pathway in vivo by testing whether NO donors can increase HR in the anesthetized rabbit independent of the autonomic nervous system. New Zealand White rabbits were vagotomized, cardiac sympathectomized, and treated with propranolol (0.3 mg/kg iv). The NO donor molsidomine (0.2 mg/kg iv) caused a progressive increase (Δ) in HR (ΔHR, 14 $\pm$ 3 beats/min; $P <$ 0.01). This effect was significantly reduced by the I_f blocker ZD-7288 (0.2 mg/kg iv; ΔHR, 2 $\pm$ 3 beats/min; P = not significant). Similar results were seen with sodium nitroprusside. The positive chronotropic effect of sodium nitroprusside (50 μM) was confirmed in the isolated working rabbit heart preparation (ΔHR, 17 $\pm$ 3 beats/min; $P < 0.01$). In conclusion, NO donors exert a small, but significant, positive chronotropic effect in vivo that is independent of the autonomic nervous system. These results are also consistent with data in sinoatrial node cells that show that NO donors increase HR by stimulating I_f.

sodium nitroprusside; molsidomine; baroreflex; rabbit

NITRIC OXIDE (NO) donors reduce arterial blood pressure (ABP) by decreasing vascular resistance and are, therefore, widely used to assess the sensitivity of the baroreceptor heart rate (HR) reflex, in particular its sympathetic component. Interpretation of these data, however, may be complicated, since nitrates can inhibit the gain of the baroreceptor-cardiac reflex (16, 18) and have additional effects on the heart itself that may be independent of reflex activation. Specifically, NO donors can reduce norepinephrine release (25) and the HR response to sympathetic nerve stimulation in vitro (4). Conversely, they can also directly increase HR in the isolated guinea pig double atria preparation by activating the hyperpolarization-activated inward pacemaking current (I_f) via a cGMP-dependent pathway (21). However, it has not been established whether NO donors have a direct effect on HR in vivo that is independent of cardiac autonomic activation.

To test this hypothesis, we assessed whether the systemic administration of the NO donors *N*-ethoxycarbonyl-3-morpholino-sydnonimine [molsidomine (Mol)] and sodium nitroprusside (SNP) can increase HR independent of cardiac autonomic reflexes in anesthetized rabbits that had been cardiac sympathectomized, vagotomized, and pretreated with propranolol. The effect of SNP on HR was also tested in the isolated (denervated) working rabbit heart preparation, in which both preload and afterload were held constant. We found that Mol and SNP can have a direct positive chronotropic effect in vivo and in vitro and that the magnitude of the tachycardia was significantly reduced by the I_f blocker 4-(*N*-ethyl-*N*-phenylamino)-1,2-dimethyl-6-(methylamino)pyrimidinium chloride (ZD-7288; Zeneca) (2, 10, 15). This suggests the involvement of an I_f-sensitive pathway. Some of the results have been previously communicated in abstract form (8).

METHODS

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) under project license PPL 30/1133.

Anesthesia

Thirty-four male New Zealand White rabbits (2.2–3.2 kg) were premedicated with Hypnorm (0.2–0.3 ml/kg im; 0.315 mg/ml of fentanyl citrate and 10 mg/ml of fluanisone; Janssen Pharmaceuticals). A surgical plane of anesthesia was induced 20 min later with halothane (2% in 100% O_2) via an Ayre's T piece with mask and bag. A 23-gauge intravenous (iv) catheter was inserted into an ear vein for administration of supplementary anesthetic (Nembutal-Urethane, 1:7) as required.

Surgery

Tracheostomy was performed, and a 3.5- or 4-mm ID. endotracheal tube (Portex) was introduced 4 cm into the trachea. Administration of halothane in O_2 was then continued through the endotracheal tube while spontaneous ventilation was maintained. Stainless steel electrodes were inserted subcutaneously into each limb to monitor electrocardiogram. Catheters (Portex) were inserted into the right femoral artery for withdrawal of blood samples and into the left carotid artery for measurement of blood pressure. The animals were partially thoracotomized to facilitate artificial ventilation (Oxford Mk II ventilator). To abolish the cardiac autonomic response to a drop in ABP, animals were vagotomized, and the aortic depressor nerves and sympathetic nerves were ligated and cut low down at the thoracic inlet. Propranolol (0.3 mg/kg iv) was also given to block any residual sympathetic activity. Efficacy of blockade was confirmed by an absence in the HR response to isoprenaline.

Measurements

Systemic ABP was measured via a saline-filled pressure transducer (SensoNor 840) and was calibrated in the midaxil-

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

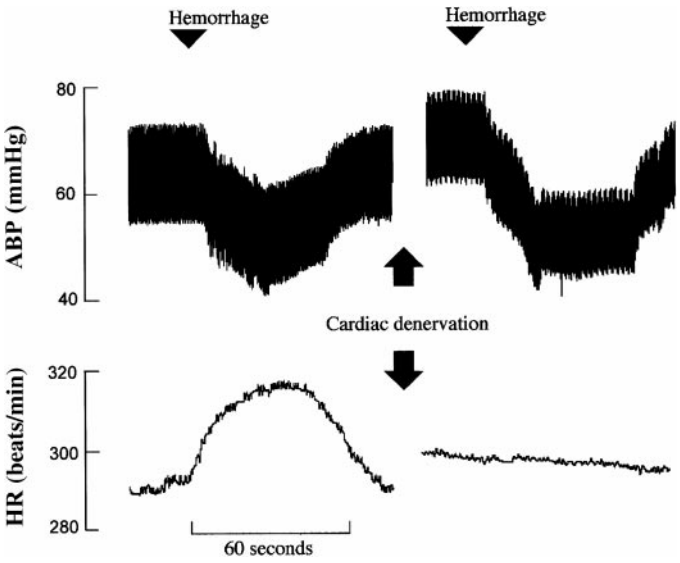

Fig. 1. *Bottom*: heart rate (HR) response to hypotension before and after cardiac autonomic denervation. *Top*: controlled withdrawal of 20 ml of arterial blood resulted in a fall in arterial blood pressure (ABP) of ~20 mmHg and a corresponding reflex tachycardia (>20 beats/min). *Right*: after vagotomy and cardiac sympathectomy, the controlled hemorrhage produced a similar drop in ABP, but the reflex tachycardia was absent.

lary line. HR traces were triggered from both ABP and electrocardiogram, respectively, and were digitally displayed. All signals were recorded onto a penwriter (MT8P, Lectromed). A Macintosh computer (Quadra 950) was used for to record and analyze data. The analog inputs were sampled at 250 Hz by a real-time data-acquisition program (Acqknowledge 3.2, model MP100, Biopac Systems), displayed on a computer monitor, and stored on compact disk. Body temperature was monitored with a rectal thermister.

Intensive Care

Animals inspired 100% O_2 throughout the experiment. Arterial blood samples (90 µl/sample) were regularly analyzed for pH and blood gases (Radiometer ABL505). Any respiratory acidosis was corrected by adjusting the frequency and/or tidal volume of the ventilator. Metabolic acidosis was corrected by iv infusion of 4.2% sodium bicarbonate solution. Fluid was replaced with a continuous iv drip of 0.9% saline (~20 ml/h). With the use of heating lamps beneath the operating table, core temperature was maintained at 38.7 ± 0.1°C.

Protocols

Efficacy of cardiac autonomic denervation was tested by performing controlled hemorrhages before and after denervation. Withdrawal of 20 ml of arterial blood resulted in a fall in ABP (~20 mmHg), with a corresponding reflex tachycardia (>20 beats/min) before denervation. Blood was then reinfused, and protocols were undertaken once HR and ABP returned to prehemorrhage values. This reflex tachycardia in response to a similar fall in ABP was abolished after cardiac denervation (Fig. 1).

Protocol 1: Effect of Mol on HR after cardiac denervation. In 11 animals, a bolus injection of the NO donor Mol was given (0.2–0.4 mg/kg iv) (22). HR and ABP were monitored for up to 30 min after administration. At this point, ZD-7288 (0.2 mg/kg iv bolus), a specific blocker of the I_f (2, 10, 15) was administered to six of these animals. This dose blocks ~75% of the HR response caused by activation of I_f (15). HR was allowed to stabilize for 20–30 min, after which a repeat dose of Mol was given and HR and ABP were monitored again over time.

Protocol 2: Effect of Mol on HR after cardiac denervation and β-adrenergic blockade. A further 11 animals were pretreated with propranolol (0.3 mg/kg iv) to block the action of circulating catecholamines, and *protocol 1* was repeated. ZD-7288 was administered to 10 of these animals.

Protocol 3: Effect on the HR response to Mol of giving I_f and β-adrenergic antagonists first. The protocol was reversed by giving ZD-7288 before Mol in a third group of rabbits ($n = 5$) that had been cardiac denervated and treated with propranolol.

Protocol 4: Effect of SNP on HR after cardiac denervation and propranolol. In another group of rabbits ($n = 7$), *protocol 2* was repeated with the use of a different NO donor, SNP (mean dose 15 $\mu g \cdot kg^{-1} \cdot min^{-1}$ iv infusion, Fresenius Injectomat-S syringe infusion pump). SNP was infused at a variable rate to maintain a constant reduction in ABP for up to 30 min. The infusion of SNP was repeated after ZD-7288. Experiments were carried out in dark conditions because SNP is sensitive to light.

Isolated working rabbit heart protocol. Hearts were removed from male New Zealand White rabbits (1.7–2.5 kg), as previously described (9). Within 2 min of removal of the heart, the aorta was cannulated and retrograde perfusion of the coronary arteries with Tyrode solution was begun in Langendorff fashion. The mode of perfusion was later switched from retrograde to anterograde perfusion to establish a working heart preparation. A preload of 10 mmHg and an afterload of 77 mmHg were set, and the heart was enclosed in a thermal chamber to ensure that temperature and humidity remained constant. After a period of stabilization, the heart was perfused with 50 µM SNP in Tyrode solution, and the effect on HR was monitored. Hearts were perfused with either 1 µM ZD-7288 or 2 mM cesium chloride (CsCl). CsCl is also a specific inhibitor of I_f (10, 21); however, by having a faster action than ZD-7288, it is better suited to the isolated working heart preparation.

Statistics

Data are shown as means ± SE. Comparisons were made by using a repeated measures one-way ANOVA; i.e., all data were compared with their own control values within the protocols, and a post hoc Scheffé's test was used. In the case of paired comparisons, a Student's paired *t*-test was used. $P < 0.05$ was accepted as statistically significant.

RESULTS

Arterial blood gases and arterial pH were well controlled throughout the experiments (Table 1).

Table 1. *pH, arterial blood gases, and HCO_3^-*

	Beginning	Middle	End
pH_a	7.38 ± 0.01	7.40 ± 0.01	7.42 ± 0.02
Pa_{O_2}, Torr	376 ± 21	412 ± 13	427 ± 13
Pa_{CO_2}, Torr	35.7 ± 1.4	31.9 ± 1.6	31.2 ± 1.4
HCO_3^-, mM	20.3 ± 0.6	19.0 ± 0.6	19.6 ± 0.7

Values are means ± SE. pH_a, arterial pH; Pa_{O_2}, arterial P_{O_2}; Pa_{CO_2}, arterial P_{CO_2}. Note that arterial blood gases, pH_a, and HCO_3^- were well controlled throughout the course of the experiments.

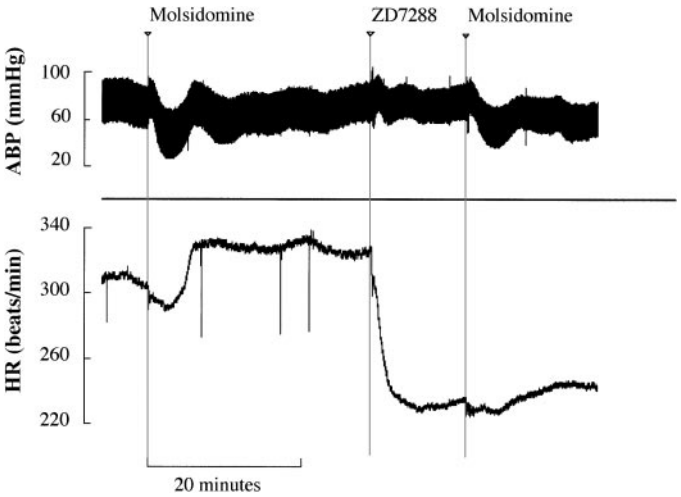

Fig. 2. Representative raw data trace of 1 experiment from *protocol 1*. ABP (*top*) and HR (*bottom*) traces show that molsidomine (Mol) caused a sustained increase in HR after its transient hypotensive effect. Increase in HR due to Mol was markedly attenuated after hyperpolarization-activated current (I_f) blockade with ZD-7288.

Protocol 1: Effect of Mol on HR After Cardiac Denervation

Figures 2 and 3 show that a bolus administration of Mol resulted in a transient fall in mean ABP [(ΔMABP), −15 ± 3 mmHg, from 71 ± 4 to 56 ± 3 mmHg]. After this hypotensive effect, ABP returned toward baseline, but HR remained elevated throughout the follow-up period. HR increased by 28 ± 6 beats/min ($P < 0.01$) with a MABP of 67 ± 3 mmHg. Administration of the blocker of the I_f, ZD-7288, caused a reduction in baseline HR of 72 ± 9 beats/min (from 303 ± 11 to 231 ± 9 beats/min; MABP, 74 ± 3 mmHg). Mol elicited a similar transient fall in ABP after ZD-7288, but the peak HR response was only increased by 8 ± 1 beats/min ($P <$ 0.05; MABP, 65 ± 3 mmHg). The rate of increase in HR in response to Mol was also markedly retarded by ZD-7288 (Fig. 3).

Protocol 2: Effect of Mol on HR After Cardiac Denervation and β-Adrenergic Blockade

Propranolol reduced baseline HR by 34 ± 8 beats/min (from 263 ± 12 to 229 ± 8 beats/min) and MABP by 7 ± 1 mmHg (from 67 ± 2 to 60 ± 2 mmHg). The HR response to Mol was diminished by ~50% in the presence of propranolol compared with the peak HR response in *protocol 1*. However, as shown in Figs. 4 and 5, Mol still caused a significant increase in HR after β-adrenergic blockade (peak ΔHR, 14 ± 3 beats/min, $P < 0.01$; MABP, 63 ± 3 mmHg). After ZD-7288 (ΔHR with ZD-7288, −33 ± 6 beats/min, from 243 ± 10 to 210 ± 6 beats/min; MABP, 67 ± 3 mmHg), the positive chronotropic effect of Mol was virtually abolished (ΔHR, 2 ± 3 beats/min, P = not significant; MABP, 71 ± 5 mmHg). Importantly, there was a significant difference between peak ΔHR responses before and after ZD-7288 ($P < 0.01$). It should be noted that the HR response did not plateau after Mol in the β-blocked animals compared with the non-β-blocked animals (see Fig. 3); therefore the maximum response was not obtained. In addition, the increase in HR was slower in the group pretreated with propranolol.

Protocol 3: Effect on the HR Response to Mol of Giving I_f and β-Adrenergic Antagonists First

The protocol was reversed by giving propranolol and ZD-7288 before Mol. Propranolol decreased HR by 20 ± 6 beats/min (from 237 ± 7 to 217 ± 8 beats/min; ΔMABP, −3 ± 2, from 61 ± 4 to 58 ± 5 mmHg). ZD-7288 decreased HR by 39 ± 6 beats/min (from 217 ± 8 to 178 ± 6 beats/min; MABP, 57 ± 4 mmHg). After I_f blockade, the bolus injection of the NO donor did not elicit a significant increase in HR (ΔHR, 6 ± 1 beats/min, P = not significant; MABP, 60 ± 7 mmHg). An example of this response is shown in Fig. 6.

Protocol 4: Effect of SNP on HR After Cardiac Denervation and Propranolol

The HR responses to SNP before and after ZD-7288 were quantitatively similar to the responses seen with Mol. After propranolol (ΔHR, −14 ± 5 beats/min, from 215 ± 9 to 201 ± 10 beats/min; ΔMABP, −11 ± 1, from

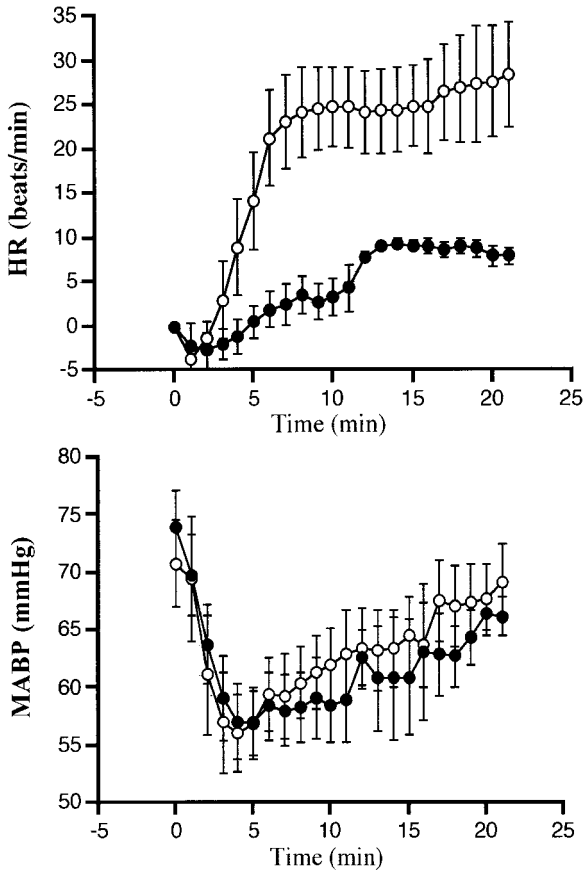

Fig. 3. Quantitative data from *protocol 1* show the mean effect of Mol (○; $n = 11$), and Mol in the presence of ZD-7288 (●; $n = 6$) on HR (*top*) and mean ABP (MABP; *bottom*) over time. Mol alone caused a significant increase in HR. Note that when MABP increased toward baseline levels, HR continued to rise. When Mol was given in the presence of ZD-7288, although a similar vascular effect occurred, the increase in HR was markedly attenuated. After 12 min in the post-ZD-7288 trace, $n = 3$. Values are means ± SE; n, no. of animals.

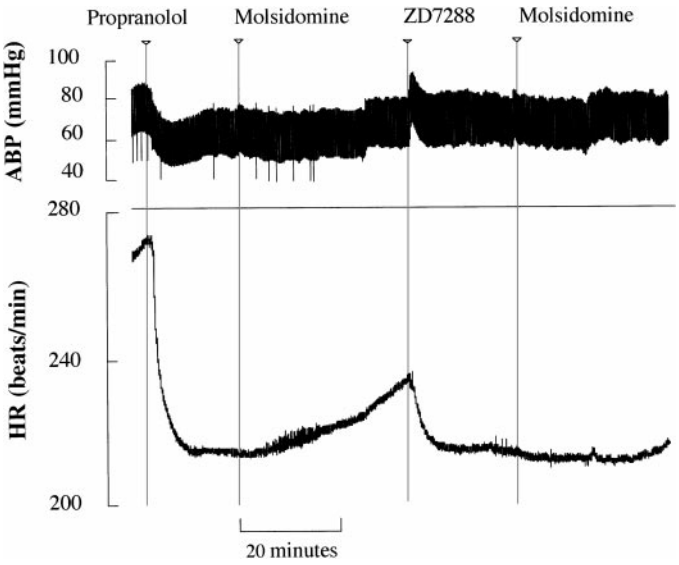

Fig. 4. Representative raw data trace of 1 experiment from *protocol 2*. ABP (*top*) and HR (*bottom*) traces show Mol increased HR, with little change in ABP. In the presence of ZD-7288, there was only a small increase in HR after ~30-min exposure to Mol. Propranolol significantly reduced HR.

72 ± 3 to 61 ± 3 mmHg) infusion of SNP resulted in a rapid and sustained fall in ABP (ΔMABP, −20 ± 3 mmHg, from 61 ± 3 to 41 ± 3 mmHg). SNP increased HR by 14 ± 4 beats/min ($P < 0.01$; MABP, 47 ± 4

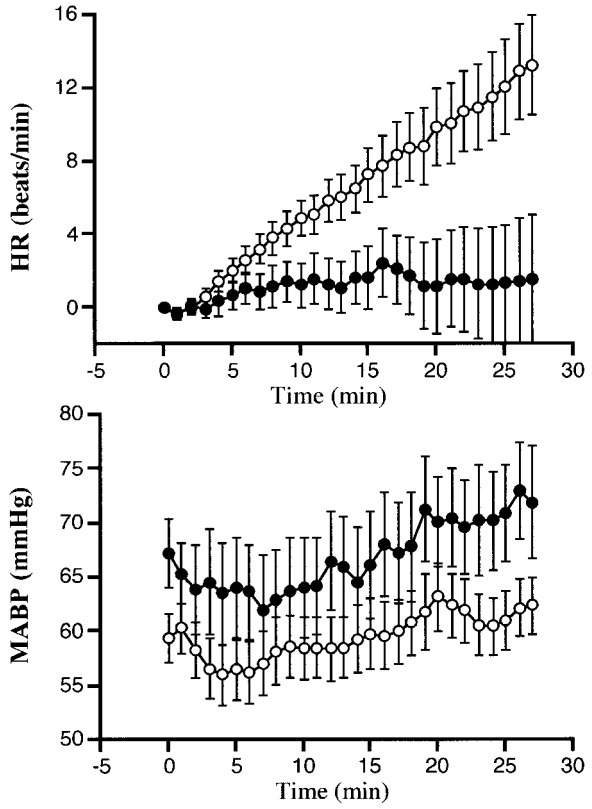

Fig. 5. Quantitative data from *protocol 2* show the mean effect of Mol (○; $n = 11$) on HR (*top*) and MABP (*bottom*) after β-blockade with propranolol. Mol produced an increase in HR that was attenuated in the presence of ZD-7288 (●; $n = 10$). Note that there was no significant change in MABP throughout. Values are means ± SE; *n*, no. of animals.

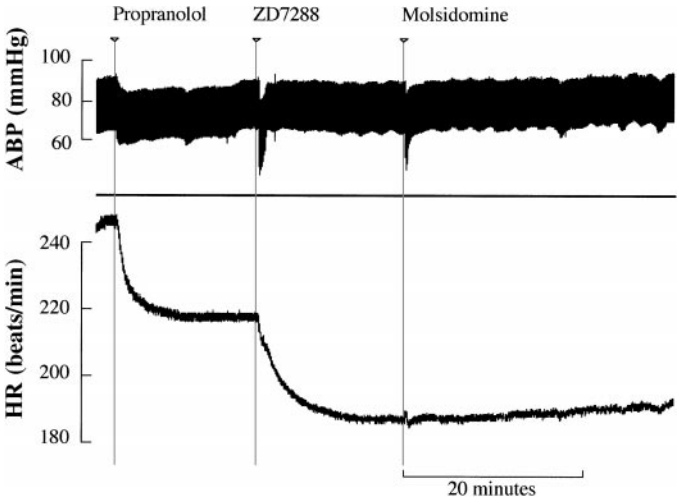

Fig. 6. Representative raw data trace from 1 experiment from *protocol 3* in which propranolol and ZD-7288 were given before Mol. *Bottom*: Mol had virtually no effect on HR in the presence of β-blockade and the I_f blocker ZD-7288.

mmHg) over the follow-up period. After administration of ZD-7288 (ΔHR −32 ± 5, from 215 ± 10 to 183 ± 5 beats/min; MABP, 66 ± 3 mmHg), the second infusion of SNP produced a similar vascular effect (ΔMABP, −19 ± 3, from 66 ± 3 to 47 ± 3 mmHg) and a smaller increase in HR (ΔHR, 7 ± 3, $P < 0.05$; MABP, 55 ± 5 mmHg). However, again there was a significant difference in peak ΔHR responses before and after ZD-7288 ($P < 0.05$).

Isolated Working Rabbit Heart Protocol

In the isolated working heart preparation with preload and afterload held constant, 50 μM SNP resulted in an increase in beating rate of 17 ± 3 beats/min ($P <$ 0.01) and a decrease in aortic flow (−26 ± 4 ml/min, from 99 ± 8 to 73 ± 9 ml/min; $n = 6$). The effect on heart rate took ~3 min to peak. Heart rate returned to baseline on washoff, although this took 36 ± 3 min (Fig. 7). In an additional set of hearts, and consistent with previous in vitro studies (21), ZD-7288 (1 mM) also significantly attenuated the positive chronotropic response to SNP (ΔHR with SNP, 15 ± 2 beats/min; and

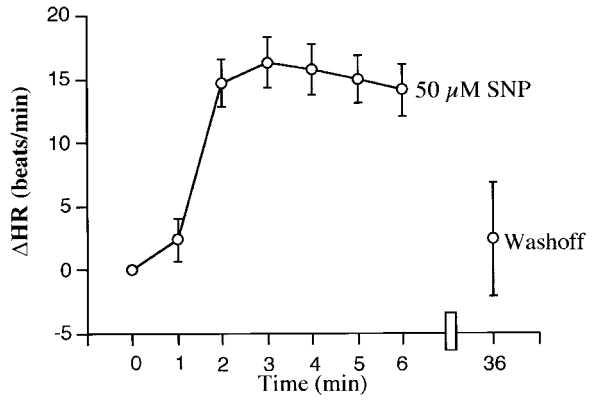

Fig. 7. Quantitative data from the isolated working rabbit heart protocol ($n = 6$) show the positive chronotropic effect of 50 μM sodium nitroprusside (SNP) on HR. Values are means ± SE; *n*, no. of animals.

ΔHR with SNP + ZD-7288, 3 ± 2 beats/min, $n = 4$; $P < 0.05$, Wilcoxon Sign test) and 2 mM CsCl (ΔHR with SNP, 19 ± 4 beats/min; ΔHR with SNP + CsCl, 3 ± 1 beats/min; $n = 9$; $P < 0.01$).

Table 2 summarizes the absolute HR responses to the NO donors and shows that these donors can significantly increase HR in the anesthetized rabbit and in the isolated working rabbit heart. Furthermore, the HR response to the NO donors is substantially reduced by the I_f blocker ZD-7288.

DISCUSSION

Our results show that *1*) NO donors have a positive chronotropic effect on HR in vivo that is independent of the cardiac innervation, *2*) SNP increases HR in the isolated working rabbit heart preparation, and *3*) the positive chronotropic response to NO donors is markedly attentuated by ZD-7288 and 2 mM CsCl, which suggests these changes are mediated via NO stimulation of an I_f-dependent pathway.

Reid (24) reported that, after bilateral sinoaortic denervation, the HR response to a 20-min infusion of SNP (~30 $\mu g \cdot kg^{-1} \cdot min^{-1}$) in conscious rabbits was abolished, although there was a trend in these data for HR to increase (see Fig. 3 in Ref. 24). A similar result is seen in the study by Ma and Long (14). Close inspection of their data also shows iv SNP increased HR by ~12 beats/min (see Fig. 1 in Ref 14). Furthermore, in heart transplant recipients who showed no evidence of autonomic reinnervation (6), inhalation of amyl nitrate (1) or infusion of SNP (12) increased HR by 9 beats/min (11%) and 13 beats/min (15%), respectively. In addition, amyl nitrate was reported to increase HR by 6% in healthy men who had been pretreated with propranolol and atropine (11).

Table 2. *Absolute mean HR values for Mol and SNP before and after I_f blockade*

	n	Control, beats/min	Plus NO Donor, beats/min
Protocol 1			
Mol	11	272 ± 10	303 ± 11†
Mol post-I_f-blockade	6	231 ± 9	240 ± 11*
Protocol 2			
Mol post-β-blockade	11	229 ± 8	243 ± 10†
Mol post-β- and I_f-blockade	10	210 ± 6	214 ± 8
Protocol 3			
Mol post-β- and I_f-blockade	5	178 ± 6	185 ± 5
Protocol 4			
SNP post-β-blockade	7	201 ± 10	215 ± 10†
SNP post-β- and I_f-blockade	7	183 ± 5	191 ± 5*
Isolated heart protocol			
SNP	6	185 ± 13	202 ± 15†

Values are means ± SE; n = no. of rabbits. Absolute heart rate (HR) values are for molsidomine (Mol) and sodium nitroprusside (SNP) before and after hyperpolarization-activated current (I_f) blockade with ZD-7288. NO, nitric oxide. *Significant difference vs. control HR, $P < 0.05$. †Significant difference vs. control HR, $P < 0.01$.

We observed a significant increase in HR following Mol (0.2–0.4 mg/kg in bolus) in anesthetized rabbits after surgical autonomic denervation of the heart. Reid (24) also showed a significant increase in arterial plasma norepinephrine after the infusion of SNP in rabbits before baroreceptor deafferentation. Because this could contribute to the increase in HR seen in our experiments, we also tested the chronotropic effect of Mol and SNP after pretreatment with propranolol. Both NO donors still caused a significant increase in HR after β-adrenergic blockade, although the rate of increase and the magnitude of effect were reduced (Figs. 3 and 5). This may reflect the complete elimination of sympathetic influences or possibly the inhibitory effect of reduction of intracellular cAMP and calcium transients by β-adrenergic blockade (19) on the activity of I_f (3). However, previous experiments in the isolated atria of the guinea pig showed that β-adrenergic blockade with nadolol (1 μM) did not affect the chronotropic effect of the active metabolite of Mol [3-morpholinosydnonimine (SIN-1)], in a range from nano- to millimolar concentrations (20). Similarly, in the isolated working rabbit heart preparation, we have observed the increase in HR due to SNP was maintained in the presence of propranolol (unpublished observation).

The chronotropic effect of SNP that we observed in vivo in the rabbit (~14 beats/min after propranolol) was smaller than that we reported in the guinea pig isolated atria (21). However, we did not perform a dose-response study in the anesthetized rabbit, and 15 $\mu g \cdot kg^{-1} \cdot min^{-1}$ of SNP were within the dose range previously employed to test the sensitivity of the arterial baroreflex in this model (29). Whereas SNP is not a prodrug and releases NO intracellularly (28), Mol is transformed into its active metabolite, SIN-1, in the liver (17, 23). The long washoff of SNP is also probably related to its intracellular action (28). Furthermore, unlike SNP, SIN-1 releases NO in the bloodstream, where it could in part be scavenged by hemoglobin or react with superoxide to form peroxynitrite. These factors make the HR response to Mol in vivo difficult to compare with the chronotropic effect of SIN-1 in vitro.

It could be speculated that the neuroendocrine response to a short period of hypotension caused by hemorrhage might have contributed to the positive chrontropic effect of NO donors in the anesthetized rabbit. However, we think this is unlikely for several reasons. First, we waited until ABP and HR returned to nearly prehemorrhage values after reinfusion of blood before measurements were taken. Second, the increase in HR with the donor was significantly reduced by the specific I_f blocker ZD-7288. Third, the positive chronotropic response to SNP was present in the isolated Tyrode-perfused heart that was devoid of circulating hormones.

Because it is virtually impossible to exclude all neural and circulatory factors that could modulate HR during in vivo experiments, we tested our hypothesis on the isolated Tyrode-perfused working rabbit heart (with preload and afterload fixed) and confirmed that the increase in HR caused by SNP was of a similar

magnitude to the increase seen in vivo. However, the increase in HR caused by 50 μM SNP was less compared with that seen in the isolated guinea pig atrial preparation (21) at the same concentration; this may reflect a species difference in response to SNP. Nevertheless, it is clear that endogenous NO has a tonic stimulatory effect on HR. NOS inhibitors decrease basal HR in vitro (5), and a similar response is seen in conscious NOS knockout mice (27).

Assessment of Baroreflex by Using Nitrovasodilators

Nitrovasodilators have been widely used to assess the sensitivity of the baroreceptor-cardiac reflex because they were thought to be direct vasodilators with no or little extravascular effects. In recent years, however, it has become evident that they exert most of their biological effects by releasing NO and that they can directly affect cardiac and autonomic function and the activity of the arterial baroreflex itself. Indeed, NO donors have been reported to decrease the gain of the baroreceptor-cardiac reflex in conscious animals (13, 18) and to suppress baroreceptor activity independent of vascular relaxation in the isolated carotid sinus preparation of the anesthetized rabbit (16). In addition, NO donors reduce norepinephrine release (25) and the HR response to sympathetic nerve stimulation in vitro (4). Conversely, NOS inhibitors enhance norepinephrine release (25) and the HR response to sympathetic nerve stimulation (4, 26). By increasing the intracellular concentration of cGMP, NO donors can also interfere with β-adrenergic signaling; this leads to an inhibition of cAMP-dependent stimulation of Ca^{2+} currents in sinoatrial cells (7). Taken together, these data indicate that NO donors may act to depress the neurally mediated HR response to a fall in ABP. Conversely, there is evidence presented here and with in vitro preparations that shows NO donors can increase HR directly.

What is the mechanism underlying the positive chronotropic effect of NO donors? Low concentrations (nanomolar to micromolar) of NO donors can increase the spontaneous beating rate in the isolated atrial preparation in guinea pigs (21). Musialek et al. (21, 22) observed that the increase in rate was mimicked by 8-bromo-cGMP, prevented by guanylate cyclase inhibitors, and suppressed after inhibition and depletion of the sarcoplasmatic Ca^{2+} stores with ryanodine and cyclopiazonic acid, respectively. In addition, the positive chronotropic effect was virtually abolished by blockers of the I_f, whereas it was not affected by the Ca^{2+} channel antagonist nifedipine. Importantly, both SNP and SIN-1 caused a time-dependent increase in I_f in rabbit sinoatrial node cells. Our results in the anesthetized rabbit are consistent with these data and suggest that, in addition to modulating sympathetic responses and baroreceptor activity, NO donors can exert a direct positive chronotropic effect which could bias the evaluation of baroreflex sensitivity by these agents. In vivo, however, this effect develops more slowly than the neurally mediated reflex HR response. Therefore it seems unlikely that this action will have a significant additional effect on HR unless NO donors are infused over minutes rather than injected as a bolus.

In summary, although our results establish that NO donors have a direct and baroreflex-independent effect on HR in vitro and in vivo, they also contribute to evidence that suggests that nitrovasodilators may not be ideal for the assessment of the baroreceptor cardiac reflex because these agents have significant extravascular effects (3).

This work was supported by the British Heart Foundation, Garfield Weston Trust (B. Casadei) and Major Stanley's Memorial Scholarship Fund (N. Hogan).

Address for reprint requests and other correspondence: D. J. Paterson, University Labortory of Physiology, Parks Rd., Oxford OX1 3PT, UK (E-mail: david.paterson@physiol.ox.ac.uk).

Received 2 November 1998; accepted in final form 23 February 1999.

REFERENCES

1. **Beck, W., C. Barnard, and V. Schrire.** Heart rate after cardiac transplantation. *Circulation* 40: 437–445, 1969.
2. **BoSmith, R. E., I. Briggs, and N. C. Sturgess.** Inhibitory actions of ZENECA ZD7288 on whole-cell hyperpolarization activated inward current (I_f) in guinea-pig dissociated sinoatrial node cells. *Br. J. Pharmacol*. 110: 343–349, 1993.
3. **Casadei, B., and D. J. Paterson.** Should we still use nitrovasodilators to test baroreflex sensitivity? *J. Hypertens*. In press.
4. **Choate, J. K., and D. J. Paterson.** Nitric oxide inhibits the positive chronotropic and inotropic responses to sympathetic nerve stimulation in the isolated guinea-pig atria. *J. Auton. Nerv. Syst*. 75: 101–108, 1999.
5. **Curtis, M. J., and R. Pabla.** Effects of NO modulation on cardiac arrhythmias in the rat isolated heart. *Circ. Res*. 77: 984–992, 1995.
6. **DeMarco, T., M. Dae, M. S. F. Yuen-Green, S. Kumar, K. Sudhir, F. Keith, R. Amidon, C. Rifkin, C. Klinski, D. Lau, E. H. Botvinick, and K. Chatterjee.** Iodine-123 metaiodobenzylguanidine scintigraph assessment of the transplanted human heart: evidence for late reinnervation. *J. Am. Coll. Cardiol*. 25: 927–931, 1995.
7. **Han, X., Y. Shimoni, and W. R. Giles.** An obligatory role for nitric oxide in autonomic control of mammalian heart rate *J. Physiol. (Lond)* 476: 309–314, 1994.
8. **Hogan, N., B. Casadei, and D. J. Paterson.** The nitric oxide donor molsidomine can increase heart rate independent of changes in arterial blood pressure in anaesthetized rabbits (Abstract). *J. Physiol. (Lond.)* 505P: 19P, 1997.
9. **Leitch, S. P., and D. J. Paterson.** Interactive effects of K^+, acidosis, and catecholamines on isolated rabbit heart: implications for exercise. *J. Appl. Physiol*. 77: 1164–1171, 1994.
10. **Leitch, S. P., C. E. Sears, H. F. Brown, and D. J. Paterson.** Effects of high potassium and the bradycardic agents ZD7288 and cesium on heart rate of rabbits and guinea-pigs. *J. Cardiovasc. Pharmacol*. 25: 300–306, 1995.
11. **Leon, D. F., J. A. Shaver, and J. J. Leonard.** Reflex heart rate control in man. *Am. Heart J*. 80: 729–739, 1970.
12. **Levine, T. B., M. T. Olivari, and J. N. Cohn.** Effects of orthotopic heart transplantation on sympathetic control mechanisms in congestive heart failure. *Am. J. Cardiol*. 58: 1035–1040, 1986.
13. **Liu, J.-L., H. Murakami, and I. H. Zucker.** Effects of NO on baroreflex control of heart rate and renal nerve activity in conscious rabbits. *Am. J. Physiol*. 270 (*Regulatory Integrative Comp. Physiol*. 39): R1361–R1370, 1996.
14. **Ma, S., and J. P. Long.** Effects of nitroglycerin on release, synthesis and metabolism of norepinephrine and activation of tyrosine hydroxylase in guinea-pigs. *Eur. J. Pharmacol*. 199: 27–33, 1991.
15. **Marshall, P. W., W. Rouse, I. Briggs, R. B. Hargreaves, S. D. Mills, and B. J. McLoughlin.** ICI D7288, a novel sinoatrial node modulator. *J. Cardiovasc. Pharmacol*. 21: 902–906, 1993.

16. **Matsuda, T., J. N. Bates, S. J. Lewis, F. M. Abboud, and M. W. Chapleau.** Modulation of baroreceptor activity by nitric oxide and *S*-nitrocysteine. *Circ. Res.* 76: 426–433, 1995.
17. **Meinertz, T., A. Brandstatter, D. Trenk, E. Jahnchen, J. Ostrowski, and W. Gartner.** Relationship between pharmacokinetics and pharmacodynamics of molsidomine and its metabolites in humans. *Am. Heart J.* 109: 644–649, 1985.
18. **Minami, N., Y. Yutaka, J.-I. Hashimoto, and K. Abe.** The role of nitric oxide in the baroreceptor-cardiac reflex in conscious Wistar rats. *Am. J. Physiol.* 269 (*Heart Circ. Physiol.* 38): H851–H855, 1995.
19. **Muller, C. A., L. H. Opie, C. W. Hamm, M. Peisach, and D. Gihwala.** Prevention of ventricular fibrillation by metoprolol in a pig model of acute myocardial ischaemia: absence of a major arrythmogenic role for cyclic AMP. *J. Mol. Cell. Cardiol.* 18: 375–387, 1986.
20. **Musialek, P., B. Casadei, and D. J. Paterson.** The chronotropic response to nitric oxide in the isolated guinea-pig atria is not affected by beta-adrenergic blockade (Abstract). *J. Physiol. (Lond)* 504 P: 83P, 1997.
21. **Musialek, P., M. Lei, H. F. Brown, D. J. Paterson, and B. Casadei.** Nitric oxide can increase heart rate by stimulating the hyperpolarization-activated inward current I_f. *Circ. Res.* 81: 60–68, 1997.
22. **Musialek, P., D. J. Paterson, and B. Casadei.** Mobilization of intracellular calcium contributes to the positive chronotropic effect of the NO donor, SIN-1, in isolated guinea-pig atria (Abstract). *Circulation* 96, *Suppl.* 1: I-357, 1997.
23. **Reden, J.** Molsidomine. *Blood Vessels* 27: 282–294, 1990.
24. **Reid, J. L.** Acute and chronic beta-receptor blockade with propranolol and the cardiovascular responses to intravenous sodium nitroprusside in the conscious rabbit. *J. Cardiovasc. Pharmacol.* 1: 403–414, 1979.
25. **Schwarz, P., R. Diem, N. J. Dun, and U. Forstermann.** Endogenous and exogenous nitric oxide inhibits norepinephrine release from rat heart sympathetic nerves. *Circ. Res.* 77: 841–848, 1995.
26. **Sears, C. E., J. K. Choate, and D. J. Paterson.** Effect of nitric oxide synthase inhibition on the sympatho-vagal control of heart rate. *J. Auton. Nerv. Syst.* 73: 63–73, 1998.
27. **Shesely, E. G., N. Maeda, H. S. Kim, K. M. Desai, J. H. Krege, V. E. Laubach, P. A. Sherman, W. C. Sessa, and O. Smithies.** Elevated blood pressures in mice lacking endothelial nitric oxide synthase. *Proc. Natl. Acad. Sci. USA* 93: 13176–13181, 1996.
28. **Stamler, J. S., and M. Feelisch.** *Methods in Nitric Oxide Research*. Chichester, UK: Wiley, 1996, p. 71–115.
29. **Wong, J., L. Chou, and I. A. Reid.** Role of AT_1 receptors in the resetting of the baroreflex control of heart rate by angiotensin II in the rabbit. *J. Clin. Invest.* 91: 1516–1520, 1993.