Nitric oxide does not modulate the hyperpolarization-activated current, $I_f$, in ventricular myocytes from spontaneously hypertensive rats

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Abstract

Objective: In sinoatrial (SA) node cells, nitric oxide (NO) exerts a dual effect on the hyperpolarization-activated current, $I_f$, i.e. in basal conditions NO enhances $I_f$ whereas in the presence of β-adrenergic stimulation it decreases it. Recent studies have shown that $I_f$ is present in ventricular myocytes from hypertrophied or failing hearts where it may promote abnormal automaticity. Since these pathological conditions are associated with increased sympathetic tone and upregulation of myocardial NO production, we set out to investigate whether $I_f$ is similarly modulated by NO in hypertrophied ventricular myocytes.

Methods: Left ventricular myocytes were isolated from 18–20-month-old spontaneously hypertensive rats (SHRs). Membrane current was measured under whole-cell or amphotericin-perforated patch-clamp conditions, at 35°C.

Results: Application of diethylamine±NO (DEA±NO, 1–100 μM) did not alter the amplitude or voltage dependence of activation of $I_f$ under basal conditions (half-activation voltage, $V_1/2$: control 82.9 ± 2.6, DEA±NO 84.0 ± 2.6 mV). Similarly, $I_f$ was not affected by the inhibition of endogenous NO production (L-NMMA, 500 μM) or guanylate cyclase (ODQ, 10 μM). Forskolin (10 μM) or isoprenaline (100 nM) elicited a positive shift in $V_1/2$ but subsequent application of DEA±NO did not further affect the properties of $I_f$.

Conclusions: Our results show that, unlike in SA node cells, in SHR ventricular myocytes basal and adrenergically stimulated $I_f$ is not modulated by exogenous NO or by constitutive NO or cGMP production.

Keywords: Adrenergic (ant)agonists; Arrhythmia (mechanisms); Hypertrophy; Ion channels; Myocytes; Nitric oxide

1. Introduction

In healthy adult hearts the hyperpolarization-activated current ($I_f$) contributes to the spontaneous diastolic depolarization of sinoatrial (SA) node cells and plays an important role in mediating the chronotropic response to β-adrenergic stimulation (for review see DiFrancesco [1]). We have previously reported that micromolar concentrations of nitric oxide (NO) donors exert a positive chronotropic effect by increasing basal $I_f$ in SA node cells via a guanylate cyclase–cGMP-mediated mechanism (NO–cGMP pathway) [2–4]. Our results were confirmed by Yoo et al. [5] who also showed that NO donors decreased $I_f$ that was prestimulated by isoprenaline via the NO–cGMP pathway. The relevance of these findings might extend beyond the regulation of heart rate since an $I_f$-like current can also be recorded in ventricular myocytes isolated from old (18–20 months) spontaneously hypertensive rats (SHR) [6,7] and from failing human hearts [8,9]. Stimulation of this current by NO or β-adrenergic agonists may promote spontaneous diastolic depolarisation and provide a substrate for abnormal automaticity in the hypertrophied or failing ventricular myocardium [10]. This may be clinically relevant since NO donors are widely used in the treatment of conditions, such as heart failure, that are associated with the occurrence of $I_f$ in the ventricular myocardium [8,9] and with an increase in cardiac sympathetic activity and circulating catecholamines [11]. Furthermore, myocardial NO synthase expression and/or activity is increased in human heart failure [12–14].
and in animal models of severe cardiac hypertrophy, such as the SHR [15,16], suggesting that regulation of the \( I_t \) current by endogenously produced NO in hypertrophied ventricular myocytes may be significant. To test this hypothesis we investigated whether basal and adrenergically stimulated \( I_t \) recorded in ventricular myocytes isolated from old SHR is regulated by endogenous NO–cGMP production or by NO donors.

2. Methods

2.1. Cell Isolation

The treatment of all animals was in accordance with the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 (HMSO). Left ventricular myocytes were isolated from old male SHR (\( \sim 18-20 \) months, 454±10 g, \( n = 48 \)) using a standard enzymatic dispersion technique as described previously [17]. At the time of isolation these animals showed no overt signs of heart failure i.e. no pleural or pericardial effusion, no ascites and the animals were not cyanosed or tachypnoeic. There was an increase in heart weight to body weight ratio (age matched WKY rats 4.3±0.01 vs. 7.02±0.3 in SHR, \( P < 0.01 \), \( n = 8 \) and \( n = 48 \), respectively) and a small but significant increase in lung-to-body weight ratio (5.2±0.15 vs. 7.4±0.3, \( P = 0.02 \)) and similar liver-to-body weight ratios. Myocytes were stored in Dulbecco’s modified Eagle medium (DMEM) at room temperature and used within 12 h.

To test for the effect of NO donors on \( I_t \) in SA node cells under the same experimental conditions used for SHR ventricular myocytes, some experiments were performed on single SA nodal cells isolated from guinea-pig hearts (600–650 g) [18]. Briefly, following collagenase perfusion (Type II), the atria were removed and dissected to reveal the SA node region, which was cut into small strips (5×2 mm) perpendicular to the crista terminalis and dispersed in 2 ml of high potassium solution. Cells were stored at 4°C and used within 6 h.

2.2. Electrophysiological techniques

Membrane current was measured at 35±1°C using either the whole-cell or amphotericin-perforated patch-clamp technique (Axopatch 200A, Axon Instruments, USA). In the whole-cell configuration microelectrode resistance was 2.4±0.1 MΩ (mean±S.E.M.) and series resistance was 4.9±0.3 MΩ (after membrane rupture); 74±1% series resistance correction and capacity compensation was routinely achieved (\( n = 43 \) ventricular myocytes). Under amphotericin-perforated patch-clamp conditions microelectrode resistance was 2.6±0.3 MΩ; series resistance was 22±3 MΩ and ~45±1% series resistance correction and capacity compensation was routinely achieved (\( n = 12 \) ventricular myocytes).

2.3. Drugs and solutions

The composition of the superfusion solution was (mM): NaCl 134; KCl 5.4; MgCl₂ 1.2; CaCl₂ 1; glucose 11.1; HEPES 5; pH 7.4 with NaOH. In ventricular myocytes \( I_t \) was recorded in the presence of (mM) 4-amino-pyridine 0.5 to block the transient outward current; BaCl₂ 5 to block the inward rectifier; NiCl₂ 5 to block the calcium current and sodium–calcium exchanger; MnCl₂ 2 and KCl 25 to enhance \( I_t \) [6,7].

The electrode filling solution contained (mM): K-aspartate 130, MgCl₂ 2, Na₂-ATP 5, CaCl₂ 5, EGTA 11, GTP 0.5, HEPES 5, pH 7.2 with KOH. For the perforated patch experiments the electrode solution contained (mM): KCl 140, NaCl 5, MgCl₂ 2, HEPES 5, amphotericin 0.26 mg/ml, pH 7.2 with KOH.

Stock solutions of the following NO donors were made fresh each day: sodium nitroprusside (SNP, \( 10^{-2} \) M in \( H_2O \)); diethylamine–NO (DEA–NO \( 10^{-2} \) M in \( H_2O \)); 3-[(±)-ethyl-2′-[(E)-hydroxymino]-5-nitro-3-hexene-carbamoyl]pyridine (NOR-4, \( 10^{-2} \) M in DMSO) and (±)-S-nitroso-N-acetylpenicillamine (SNAP, \( 10^{-2} \) M in \( H_2O \)). Forskolin (\( 10^{-2} \) M in DMSO) was used as an activator of adenylate cyclase; L-[1,2,4]oxadiazolo[4,3-a]quinoxalin-S-nitroso-L-arginine, monoacetate salt (L-NMMA, 10 M in \( H_2O \)) was used as an inhibitor of NO-sensitive guanylate cyclase and \( N\)-monomethyl-L-arginine, monoacetate salt (1-NMMA, \( 10^{-4} \) M in \( H_2O \)) was used as a nitric oxide synthase (NOS) inhibitor. DEA–NO, SNAP, NOR-4, forskolin, ODQ and L-NMMA were purchased from Calbiochem and all other chemicals from Sigma.

2.4. Experimental protocols

Hyperpolarization-activated inward current (\( I_t \)) was elicited by step hyperpolarizations from a holding potential of \(-40\) mV to various test potentials (range \(-50 \) to \(-140\) mV in \(-10\) mV increments), the initial duration of the step hyperpolarisation was 3 s and was decreased by 200 ms on successive hyperpolarisations [6,7]. At the end of each step hyperpolarisation, membrane voltage was stepped to +20 mV (400 ms) before returning to the holding potential. \( I_t \) amplitude was measured as the difference between the instantaneous current recorded at the start of the pulse and the steady-state current recorded at the end of the step hyperpolarisation [6,7]. Specific conductance (\( g \)) was calculated from \( g = I/(V_m - V_{rev}) \) where \( I \) is the current amplitude, \( V_m \) is membrane voltage and \( V_{rev} \) the reversal potential. For each cell, the reversal potential of \( I_t \) was calculated from analysis of tail currents [7,9] (mean value \(-21.4±0.7\) mV, \( n = 57 \)). Steady-state activation curves for each cell were constructed by plotting normalised conductance (\( g/g_{max} \)) as a function of membrane voltage (\( I_t \) is
reported to be fully activated at −120 mV [6] see Fig. 1, thus g measured at −120 mV was used as an approximation of \( g_{\text{max}} \). Data points were fitted to a Boltzmann function \( g / g_{\text{max}} = 1 / [1 + \exp ((V_m - V_h)/k)] \), where \( g / g_{\text{max}} \) is the normalised conductance recorded at membrane voltage \( V_m \), \( V_h \) is the potential at which the current is half maximally activated and \( k \) is the slope factor. Cell membrane capacitance (\( C_m \)) was measured by applying a −10 mV pulse (from a holding potential of −40 mV, 50 mS duration). The current transient was fitted to a mono-exponential; the time course of decay of the current transient is related to the cell capacitance [8].

2.5. Statistics

Data are expressed as mean±S.E.M. and \( n \) indicates the number of cells used. Student’s paired \( t \)-test or one-way factorial ANOVA was used to assess drug effects and statistical significance was assessed at the 0.05 level. Multiple group comparisons were made using one-way ANOVA.

3. Results

3.1. Properties of \( I_f \) recorded in single ventricular myocytes isolated from SHR

Fig. 1A shows a family of hyperpolarization-activated current records taken from a single SHR ventricular myocyte in the absence and presence of caesium (\( \text{Cs}^+ \), 5 mM). Currents were elicited by the voltage protocol shown in Fig. 1B. Currents elicited by a step hyperpolarization to −90 mV are superimposed in Fig. 1C. Caesium reduced the amplitude of \( I_f \) (at −90 mV) to 14±3% (\( n = 19 \)) of control values. Conductance–voltage relationship curves for mean data (\( n = 43 \)) are shown in Fig. 1D. Conductance at −120 mV (−\( g_{\text{max}} \)) was 54.6±5.9 pS/pF, the voltage at which half-activation was achieved (\( V_h \)) was −90.9±1.8 mV and the slope (\( k \)) was 11.0±0.7 mV.

3.2. Effect of NO donors on basal \( I_f \) in ventricular myocytes

Fig. 2A shows typical \( I_f \) records taken from a single SHR ventricular myocyte under whole-cell patch-clamp conditions before and after the application of DEA–NO (1 \( \mu \)M for 5 min). Superimposed current records elicited by a step hyperpolarization to −90 mV (Fig. 2B) show that application of DEA–NO does not alter the amplitude of \( I_f \) (control −1.93±0.29 pA/pF, DEA–NO −1.96±0.42 pA/pF; \( P = \text{NS}, n = 12 \)). Similarly, the voltage dependence of activation of \( I_f \) is not altered by DEA–NO (\( V_h \); control −82.9±2.6 mV; DEA–NO −84.0±2.6 mV, \( P = \text{NS}, \) Fig. 2C).

Evidence indicates that the effects of NO donors on cardiac pacemaking and contractile function can be biphasic and concentration dependent [2,19,20], however application of a higher concentration of DEA–NO (100 \( \mu \)M, 5 min) also failed to alter the properties of \( I_f \) (amplitude at −90 mV: control −2.8±0.5 pA/pF; DEA–NO −2.5±0.4 pA/pF, \( P = \text{NS}, V_h \); control −85.5±2.2 mV; DEA–NO −87.6±2.5 mV, \( P = \text{NS}, n = 6 \)). Application of two other structurally different NO donors (SNP 10 \( \mu \)M, \( n = 20 \) or NOR-4 1 \( \mu \)M, \( n = 4 \)) produced qualitatively similar results (data not shown).

To test whether the absence of NO-mediated regulation of \( I_f \) in ventricular myocytes may be due to the dialysis of the intracellular milieu that occurs using the whole-cell configuration, we also examined the effect of NO on \( I_f \) using the perforated patch-clamp technique. Under these conditions the properties of \( I_f \) were similarly unchanged by
I and the soluble guanylate cyclase inhibitor ODQ on the conductance of the application of 1 μM 5'-AMP (see Methods) or DMEM supplemented with either 500 μM 2-NMMA or 10 μM ODQ and $I_t$ was measured in equal numbers of cells per group. Fig. 3 shows that the basal properties of $I_t$ were not altered by inhibition of either NOS or guanylate cyclase. The amplitude (Fig. 3A and B) and voltage dependence (Fig. 3C) of $I_t$ did not differ between the three groups ($V_h$: control $-90.1±1.8$ mV, $n=18$; 2-NMMA $-94.1±2.9$ mV, $n=18$; ODQ $-88.8±1.1$ mV, $n=18$, ns).

### 3.4. Effect of forskolin and NO on the properties of $I_t$ in ventricular myocytes

The effect of NO donors on $I_t$ in SA node cells is dependent on [cAMP], i.e. NO donors increase $I_t$ in basal conditions [2] but they inhibit the current in the presence of β-adrenergic stimulation [5]. To test whether these findings apply to the $I_t$ recorded in SHR ventricular myocytes, we studied the effect of 1 μM DEA–NO after prestimulation of adenylate cyclase by forskolin. Application of forskolin (10 μM, for 3 min) increased $I_t$ amplitude (for example, at $-80$ mV forskolin increased current amplitude from $-1.5±0.4$ pA/pF to $-2.7±0.6$ pA/pF, $n=7$, $P<0.005$, Fig. 4A and B) and shifted the voltage dependence of activation by 18.8±3.7 mV to more positive potentials (from $-83.6±2.4$ mV to $-64.6±4.0$ mV, $n=7$, $P<0.005$, Fig. 4C). Subsequent application of DEA–NO did not significantly alter the value of $V_h$ ($-66.3±5.8$ mV). Conductance (at $-120$ mV) was not significantly different between control 54.9±8.1 pS/pF, forskolin 55.5±9.9 pS/pF and forskolin + DEA–NO 52.8±11.6 pS/pF ($n=7$, $P=0.77$, 1-way ANOVA).

Qualitatively similar findings were observed when SNAP (1 μM) was applied to cells prestimulated with forskolin ($V_h$: control $-76.4±2.9$ mV; forskolin $-64.3±0.4$ mV and forskolin + SNAP $-64.9±1.9$ mV, $n=2$) or with isoprenaline (100 nM) ($V_h$: control $-82.4±1.5$ mV; isoprenaline $-73.4±5.2$ mV and isoprenaline + SNAP $-72.3±4.4$ mV, $n=3$). When applied subsequent to an NO donor, forskolin increased $I_t$ amplitude by a similar amount (control $-2.1±0.2$; NO donor $-2.0±0.2$; forskolin $-2.6±0.3$ pA/pF at $-90$ mV, $P<0.001$, $n=13$).

### 3.5. Effects of NO on basal $I_t$ in single SA node cells

In contrast to the findings in ventricular myocytes, application of DEA–NO (1 μM, 5 min) increased the amplitude of $I_t$ recorded in single SA node cells under perforated patch-clamp conditions (Fig. 5A). Although DEA–NO increased $I_t$ amplitude elicited by a step hyperpolarization to $-70$ mV (Fig. 5B) from $-2.5±0.7$ to $-3.8±1.0$ pA/pF ($n=5$, $P<0.02$) the amplitude and conductance of $I_t$ elicited at $-120$ mV was not altered (amplitude: control $-14.2±3.2$ pA/pF; DEA–NO $-14.8±2.7$ pA/pF, $n=5$, ns; conductance: control 149.7±26.4 pS/pF; DEA–NO 162.4±25.3 pS/pF, ns).

**Fig. 2.** Effect of the NO donor DEA–NO on $I_t$ recorded in ventricular myocytes. (A) Typical currents recorded from a single ventricular myocyte at $-60$, −70, −80, −90 and −100 mV in the absence and presence of 1 μM DEA–NO. For clarity, currents have been truncated immediately prior to the depolarisation capacity transient. (B) Superimposed currents (at −90 mV) recorded under control conditions and in the presence of DEA–NO. (C) Mean normalised conductance–voltage relationships in the absence and presence of DEA–NO ($n=12$).

### 3.3. Effect of L-NMMA and ODQ on the properties of $I_t$ in ventricular myocytes

To assess whether endogenous NO or cGMP production can constitutively activate $I_t$ in SHR ventricular myocytes, we investigated the effects of the NOS inhibitor L-NMMA and the soluble guanylate cyclase inhibitor ODQ on the properties of $I_t$. Myocytes from the same isolation were incubated for 30 min in control media (DMEM, see Methods) or DMEM supplemented with either 500 μM L-NMMA or 10 μM ODQ and $I_t$ was measured in equal numbers of cells per group. Fig. 3 shows that the basal properties of $I_t$ were not altered by inhibition of either NOS or guanylate cyclase. The amplitude (Fig. 3A and B) and voltage dependence (Fig. 3C) of $I_t$ did not differ between the three groups ($V_h$: control $-90.1±1.8$ mV, $n=18$; L-NMMA $-94.1±2.9$ mV, $n=18$; ODQ $-88.8±1.1$ mV, $n=18$, ns).
Fig. 3. Effect of inhibition of NOS and guanylate cyclase on \( I \), recorded in ventricular myocytes. (A) Typical current records taken from single ventricular myocytes preincubated in control solution, in 500 μM L-NMMA or in 10 μM ODQ. Currents shown were elicited by step-hyperpolarisations from −50 to −110 mV in 10-mV steps. (B) Mean \( I \) density (at −90 mV) recorded under control conditions and after 500 μM L-NMMA or 10 μM ODQ. (C) Mean normalised conductance–voltage relationships in control conditions (○) and after preincubation with L-NMMA (□) or ODQ (△) (n=18 for each group).

Fig. 4. Effect of forskolin and DEA–NO on \( I \), recorded in SHR ventricular myocytes. (A) Currents recorded from a ventricular myocyte (at −50, −60, −70, −80, −90 and −100 mV) under control conditions (left) and in the presence of 10 μM forskolin (centre) or forskolin plus 1 μM DEA–NO (right). For clarity, currents have been truncated immediately prior to the depolarisation capacity transient. (B) Superimposed current records elicited by a step hyperpolarisation to −80 mV under these conditions. (C) Mean normalised conductance–voltage relationships (n=7).
constitutive myocardial NO or cGMP production. Conversely, increasing [cAMP], with forskolin or isoprenaline increased the amplitude of \( I_f \) by shifting the voltage dependence of the current toward more positive potentials. In summary, our data suggest that, in contrast to SA node cells, basal \( I_f \) is not enhanced by NO in hypertrophied ventricular myocytes. Importantly, however, NO does not oppose the cAMP-mediated increase in \( I_f \) in this preparation. This may contribute to the enhanced arrhythmogenic effect of \( \beta \)-adrenoceptor stimulation in old SHR [21].

4.1. \( I_f \) in ventricular myocytes

Our data confirm that a caesium-sensitive hyperpolarization-activated inward current that activates within the physiological range of membrane potentials is present in ventricular myocytes isolated from old SHRs [6,7]. Previous studies have shown that \( \beta \)-adrenergic stimulation increases \( I_f \) activity in ventricular myocytes from SHRs [7,22] and failing human hearts [9]. Similarly, we found that stimulation of adenylate cyclase by forskolin increased the amplitude of \( I_f \) by shifting the voltage dependence of activation by \( \sim 18 \) mV towards more positive potentials without increasing maximal conductance. Although this is greater than the 11 mV maximal shift in \( V_h \) caused by cAMP in excised macropatches from the rabbit SA node [23], it is similar to the shift caused by noradrenaline (17 mV in the presence of \( G_i \) inhibition) in SHR ventricular myocytes [22]. Taken together these data show that \( I_f \) in ventricular myocytes is enhanced by cAMP, presumably via mechanism(s) similar to those described in the SA node (i.e. direct binding of cAMP to the channel increasing open channel probability [1,23]).

4.2. Contrasting effect of NO on \( I_f \) in SA node cells and ventricular myocytes

Micromolar concentrations of NO donors increase basal \( I_f \) in SA node cells by shifting the voltage dependence of activation of the current toward more positive potentials (Fig. 5). Yoo et al., however, showed that in the presence of \( \beta \)-adrenergic stimulation NO donors inhibit \( I_f \) by shifting the voltage dependence of activation in the opposite direction [5]. In contrast with the findings in SA node cells, we observed no effect of NO donors on basal and forskolin-stimulated \( I_f \) in SHR ventricular myocytes. The reason(s) for the differential regulation of \( I_f \) by NO between SA node cells and ventricular myocytes is at present unclear. We considered the possibility that under whole-cell patch-clamp conditions, dialysis of the intracellular milieu and calcium buffering may have attenuated the response to exogenous NO. However, although we used the perforated patch-clamp technique to obviate these methodological problems, the properties of \( I_f \) remained unaltered by the application of NO donors, thus excluding this possibility.
Several reports show that NOS expression and activity are upregulated in the failing human heart [12–14] and in the hypertrophied SHR myocardium [15,16] suggesting that endogenous NO production within cardiac myocytes may be increased. To test whether constitutive activation of \( I_f \) by endogenous NO may be maximal, thereby preventing further stimulation of \( I_f \) by NO donors, we investigated the effect of inhibitors of NOS (NOS) or guanylate cyclase on basal \( I_f \). Our findings, however, showed that \( \text{L-NAME} \) and ODQ do not alter the basal properties of \( I_f \), indicating that the current is not constitutively stimulated by endogenous NO or cGMP production in SHR ventricular myocytes.

In SA node cells, NO-mediated regulation of \( I_f \) is cGMP-dependent [2,5]. The mechanism(s) by which a NO-mediated increase in [cGMP] can affect \( I_f \) include direct activation of the channel by cGMP binding [23] and regulation of cAMP breakdown through the cGMP-dependent phosphodiesterases of cAMP [4,5]. Thus, impairment of NO–cGMP downstream signalling in the severely hypertrophied myocardium may account for the lack of NO-mediated regulation of \( I_f \) in SHR ventricular myocytes. Consistent with this idea, Ito et al. [24] showed that SNP and 8-bromo-cGMP suppressed systolic contraction and increased diastolic cell length in normal but not in hypertrophied myocytes. Similarly, Matter et al. [25] reported that intracoronary infusion of SNP in patients with severe aortic stenosis and LV hypertrophy has no effect on LV inotropy and relaxation (unlike in subjects with a normal heart [26]). Also, increased myocardial eNOS protein expression and NO production in SHRs are not associated with a higher cGMP content or with a greater decrease in myocardial cGMP concentration after NOS inhibition when compared with normotensive rats [15]. As expression of soluble guanylate cyclase is not suppressed in the SHR ventricular myocardium [27], desensitisation of this enzyme to activation by NO or rapid scavenging of NO by excess superoxide production [28] may contribute to the reduction in NO bioactivity in the SHR myocardium, despite enhanced NOS activity. Thus, NO and cGMP synthesis may be uncoupled in the hypertrophic myocardium and suppression of cGMP downstream signalling may occur as the severity of LV hypertrophy increases, explaining, at least in part, the absence of NO-mediated regulation of \( I_f \) in the old SHRs.

Our findings, however, may also reflect different sensitivity to cGMP between the SA node and ventricular \( f \) channels. Indeed, hyperpolarisation-activated, cyclic nucleotide gated channels with the general properties of neuronal or cardiac \( I_f \) can be encoded for by at least four different genes (hyperpolarization-activated cation channel, HCN 1–4) (reviewed in [29]). Both HCN-2 and HCN-4 are expressed in the rat ventricular myocardium with a ratio of 13:1 in the adult and 5:1 in the neonate [30] and HCN-2 mRNA expression has been found to be positively correlated with the severity of left ventricular hypertrophy in the SHRs [31]. In contrast, the dominant isoforms in the SA node are HCN-4 (81%) and HCN-1 (18%) [30,32]. These isoforms appear to have differing sensitivities to cAMP, e.g. the shifts in \( V_h \) of mouse HCN-2 and HCN-1 in the presence of 1 mM cAMP are +16 mV and +2 mV, respectively [33,34]. Although the cGMP sensitivity of the HCN channels has not been systematically investigated, it is conceivable that different HCN ratios may change the \( I_f \) response to cyclic nucleotides and thus to NO.

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