Norepinephrine Release from the Ischemic Heart Is Greatly Enhanced in Mice Lacking Histamine H₃ Receptors

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ABSTRACT

We previously reported that histamine H₃ receptors (H₃Rs) are present in cardiac sympathetic nerve endings (cSNE) of animals and humans, where they attenuate norepinephrine (NE) release in normal and hyperadrenergic states, such as myocardial ischemia. The recent creation of a transgenic line of mice lacking H₃R provided us with the opportunity to assess the relevance of H₃Rs in the ischemic heart. We isolated SNE from hearts of wild-type (H₃R+/+) and knockout (H₃R⁻/⁻) mice and found that basal NE release from H₃R⁻/⁻ cSNE was ~60% greater than that from H₃R+/+ cSNE. NE exocytosis evoked by K⁺-induced depolarization of cSNE from H₃R⁻/⁻ mice was attenuated by activation of either H₃R or adenosine A₁ receptors (A₁Rs). In contrast, NE release from cSNE of H₃R⁻/⁻ was unaffected by H₃R agonists, but it was still attenuated by A₁R activation. When isolated mouse hearts were subjected to ischemia for 20 min, NE overflow into the coronaries was 2-fold greater in the H₃R⁻/⁻ hearts than in those from H₃R+/+ mice. Furthermore, whereas stimulation of H₃R or A₁R reduced ischemic NE overflow from H₃R⁻/⁻ hearts by 50%, only A₁R, but not H₃R activation, reduced NE release in H₃R⁻/⁻. Our data demonstrate that NE release from cSNE can be modulated by various heteroinhibitory receptors (e.g., H₃R and A₁R) and that H₃Rs are particularly important in modulating NE release in myocardial ischemia. Inasmuch as excessive NE release is clinically recognized as a major cause of arrhythmic cardiac dysfunction, our findings reveal a significant cardioprotective role of H₃R on cSNE.

Sympathetic overactivity accompanied by excessive norepinephrine (NE) release is clinically recognized as a major cause of arrhythmic cardiac dysfunction in myocardial ischemia (Braunwald and Sobel, 1988; Kurz et al., 1991; Dart and Du, 1993; Kubler and Strasser, 1994; Benedict et al., 1996). Indeed, myocardial infarction is often accompanied by arrhythmias with high morbidity and mortality (Braunwald and Sobel, 1988; Schomig et al., 1995; Airaksinen, 1999). Sympathetic overactivity and excessive NE release increase metabolic demand, thereby aggravating the primary ischemia and initiating a vicious cycle that can culminate in further myocardial damage and severe cardiac failure (Kubler and Strasser, 1994). Moreover, once released, NE enhances intracellular Ca²⁺ by increasing its influx through voltage-dependent channels, mobilizing it from intracellular stores and favoring its inward transport by the Na⁺/Ca²⁺ exchanger. Ca²⁺ overload eventually results in dysrhythmia and uncoordinated myocyte contraction (Levi and Smith, 2000). Therefore, negative modulation of NE release from cardiac sympathetic nerves is a crucial protective mechanism.

We have shown that activation of histamine H₃ receptors (H₃Rs) on cardiac sympathetic nerve endings (cSNE) negatively modulates NE release from ischemic hearts and attenuates the severity of associated ventricular arrhythmias (Levi and Smith, 2000). H₃Rs are but one of several classes of prejunctional heteroinhibitory receptors (Imamura et al., 1996), and their efficacy in myocardial ischemia models has been tested to date only by pharmacological antagonism of their effects (Levi and Smith, 2000). The availability of a newly created transgenic line of mice lacking H₃R (Toyota et al., 2002) permits us to compare myocardial ischemia in the absence and presence of H₃R and, thus, to evaluate the relevance of H₃R as a basic modulatory mechanism of ischemic NE release. We report the novel finding that hearts with H₃R deletion release more than twice as much NE when subjected to ischemia than hearts with intact H₃R. This finding underscores the relevance of H₃R as a major cardioprotective mechanism in myocardial ischemia.

Materials and Methods

Generation of Histamine H₃R⁻/⁻ Mice. H₃R⁻/⁻ knockout mice were generated, and deletion was verified with radioligand binding...
and pharmacological challenge as described previously (Toyota et al., 2002).

**NE Release from Ischemic Mouse Hearts.** Male wild-type H\(_3\)R\(^{+/+}\) (body weight, 26.6 ± 0.4 g; heart weight, 141 ± 3 mg; n = 49) and knockout H\(_3\)R\(^{-/-}\) mice (body weight, 27.2 ± 0.4 g; heart weight, 144 ± 2 mg; n = 35) were killed by cervical dislocation under light anesthesia with CO\(_2\) vapor in accordance with institutional guidelines. The ribcage was dissected away, and the heart was rapidly excised, freed from fat and connective tissue and transferred to a Langendorff apparatus. The aorta was cannulated with a flanged 18-gauge stainless-steel needle. Spontaneously beating hearts were perfused through the aorta in a retrograde mode at a constant pressure of 100 cm of H\(_2\)O with modified Krebs-Henseleit buffer (KHB) containing 120 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl\(_2\), 1.2 mM MgSO\(_4\), 1.2 mM KH\(_2\)PO\(_4\), 25 mM NaHCO\(_3\), 11 mM glucose, and 0.5 mM EDTA. The perfusion fluid was equilibrated with 95% O\(_2\)/5% CO\(_2\) at 37°C to give a pH of 7.4. After a 30-min stabilization period, normothermic ischemia was induced by perfusing hearts for 20 min with glucose-free KHB equilibrated with 95% N\(_2\) and 5% CO\(_2\) and containing the reducing agent sodium dithionite (final concentration of 0.25 mM). Hearts receiving drug treatment were treated for 15 min before induction of ischemia. The coronary effluent was collected into tubes. In the preischemic and ischemic periods, tubes were replaced every 5 min. The volume of effluent collected for each period was weighed and subsequently analyzed for NE content. All drugs were added to the perfusion solution. NE was assayed in the coronary perfusate by high-pressure liquid chromatography with electrochemical detection (Silver et al., 2002).

**NE Release from Cardiac Synaptosomes.** Cardiac synaptosomes were isolated as described previously for the guinea pig (Seyedi et al., 1997; Silver et al., 2002). Briefly, hearts suspended in 200 ml H\(_2\)O \(H\(_{\text{3R}}^{+/-}\) and 20 H\(_2\)O \(H\(_{\text{3R}}^{-/-}\) mice were excised as described above and transferred to a Langendorff apparatus. Spontaneously beating hearts were perfused through the aorta for 15 min at constant pressure (100 cm of H\(_2\)O) with modified KHB at 37°C saturated with 95% O\(_2\) and 5% CO\(_2\), pH 7.4. This procedure ensured that no blood was indistinguishable from that observed in the same preparation from the guinea pig heart (Seyedi et al., 1997), whose synaptosomal composition had been ascertained by electron microscopy (R. Levi and N. Seyedi, unpublished observations).

**Statistics.** Values are expressed as the mean percentage increase above basal NE release (synaptosomes) or as absolute values for NE overflow (isolated hearts) ± S.E.M. Analysis by one-way ANOVA was used, followed by post hoc testing (Dunnett’s test). A p value of <0.05 was considered statistically significant.

**Results**

**Exocytosis of Endogenous Norepinephrine from Cardiac Sympathetic Nerve Terminals.** As we did previously with human (Imamura et al., 1995), guinea pig (Seyedi et al., 1997), and dog (Seyedi et al., 1996) cardiac tissue, we first assessed whether the mouse heart harbors H\(_3\) inhibitory heteroreceptors located prejunctionally on SNE. For this, we studied the action of the selective H\(_3\)R agonist imetit (Garraghty et al., 1992) directly on SNE (cardiac synaptosomes) isolated from wild-type (H\(_2\)O \(H\(_{\text{3R}}^{-/-}\)) mouse hearts. As shown in Fig. 1, A and B, depolarization of mouse cSNE with 100 mM K\(^+\) resulted in a 20 to 30% increase in NE release above the basal level of 0.76 ± 0.11 pmol/mg (mean ± S.E.M.; n = 20). When cSNE were pretreated with imetit (100 nM), NE release in response to K\(^+\)-induced depolarization was reduced by ~50%. This effect of imetit was prevented by pretreatment with the selective H\(_3\)R antagonist thioperaamide (Arrang et al., 1987) (300 nM) (Fig. 1A). We also determined the presence of other prejunctional inhibitory heteroreceptors in the cSNE of H\(_2\)O \(H\(_{\text{3R}}^{-/-}\) mice. As shown in Fig. 1B, the selective adenosine A\(_1\) receptor (A\(_1\)R) agonist N\(_6\)-cyclopentyladenosine (CPA; 300 nM) (Barrett et al., 1994) decreased K\(^+\)-induced NE release by ~70%. This effect of CPA was prevented by pretreatment with the selective A\(_1\)R antagonist 3-cyclopropyl-1,3-dipropylxanthine (DPCPX) (300 nM) (Haleen et al., 1987) (Fig. 1B).

As shown in Fig. 1, C and D, K\(^+\)-induced depolarization of cSNE isolated from hearts of mice lacking H\(_2\)R (H\(_2\)R\(^{-/-}\)) resulted in a ~25 to 35% increase in NE release above the basal level of 1.25 ± 0.06 pmol/mg (mean ± S.E.M.; n = 20). Notably, this basal level was ~60% greater than that for synaptosomes isolated from H\(_2\)O \(H\(_{\text{3R}}^{+/-}\) mouse hearts (p < 0.01). Contrary to its action on SNE from H\(_2\)O \(H\(_{\text{3R}}^{+/-}\) mouse hearts, imetit failed to modify the K\(^+\)-induced NE release in SNE isolated from H\(_2\)O \(H\(_{\text{3R}}^{-/-}\) mouse hearts (Fig. 1C). However, in H\(_2\)R\(^{-/-}\) cSNE, activation of A\(_1\)R with CPA still caused a ~70% reduction in K\(^+\)-induced NE release, which was prevented by pretreatment with DPCPX (Fig. 1D). This suggested that although H\(_3\)R-mediated modulation of NE exocytosis had been deleted in H\(_2\)R\(^{-/-}\) mouse hearts, A\(_1\)R-mediated modulation was preserved.

**Release of Endogenous Norepinephrine from the Ischemic Heart.** Inasmuch as these findings indicated the absence of inhibitory H\(_2\)R on cSNE of H\(_2\)R\(^{-/-}\) mice, we next questioned whether such an absence might influence NE release in myocardial ischemia, given that H\(_2\)R are known to negatively modulate NE release in this condition (Levi and Smith, 2000). When hearts from either H\(_2\)R\(^{+/-}\) or H\(_2\)R\(^{-/-}\) mice were excised and perfused in a Langendorff apparatus in normoxic conditions, NE overflow into the coronary effluent was below the detection threshold (data not shown). When hearts from H\(_2\)R\(^{-/-}\) mice were perfused for 20 min in...
ischemic conditions (glucose-free buffer containing the reducing agent sodium dithionite and equilibrated with 95% N₂ and 5% CO₂), total NE overflow increased to ~400 pmol/g (Fig. 2A). The NE transporter inhibitor desipramine (100 nM) markedly inhibited (~50%) this increase in overflow (Fig. 2A), indicating that ischemic NE release was carrier-mediated; that is, NE was carried out of cSNE by the NE transporter in a reversed mode of action (Levi and Smith, 2000). In hearts perfused with imetit (100 nM), ischemic NE overflow was reduced by ~40%. This effect was abolished in the presence of thioperamide (300 nM). In fact, with thioperamide, either alone or combined with imetit, ischemic NE overflow was ~35% greater than that in control conditions (Fig. 2A). In hearts perfused with CPA (100 nM), ischemic NE overflow was reduced by ~50%. This effect was abolished in the presence of DPCPX (100 nM).

In marked contrast, when hearts from H₃R⁻/⁻ mice were perfused for 20 min in ischemic conditions, total NE overflow was more than 2-fold greater than in H₃R⁺/⁺ mouse hearts (p < 0.01) (Fig. 2B). As in H₃R⁺/⁺ hearts, desipramine (100 nM) markedly inhibited (~65%) this increase in overflow (Fig. 2B), indicating that ischemic NE release in H₃R⁻/⁻ hearts was also carrier-mediated. However, neither imetit nor thioperamide modified ischemic NE overflow in H₃R⁻/⁻ hearts (Fig. 2B). Similar to its action on H₃R⁺/⁺ hearts, CPA (100 nM) again reduced ischemic NE overflow by ~50%, an effect that was prevented by DPCPX (100 nM) (Fig. 2B).

Discussion

In protracted myocardial ischemia, metabolic acidosis develops in SNE, leading to activation of the Na⁺/H⁺ exchanger and, thus, to an increase in intraneuronal Na⁺ concentration.

Fig. 1. Exocytotic NE release from mouse heart sympathetic nerve endings (cardiac synaptosomes) depolarized with 100 mM K⁺. Bars indicate the mean percentage increases in NE release above basal levels (± S.E.M.). Basal NE release was 0.76 ± 0.11 and 1.25 ± 0.06 pmol/mg protein for H₃R⁺/⁺ and H₃R⁻/⁻, respectively (n = 20 + 20; p < 0.01). Drugs were administered at the following concentrations: imetit, 100 nM; thioperamide, 300 nM; CPA, 300 nM, and DPCPX, 300 nM. These data show that the H₃R-mediated attenuation of NE exocytosis is lost in the synaptosomes of H₃R⁻/⁻ mice, whereas the modulatory activity of the adenosine A₁R-agonist CPA is preserved. A total of 40 mouse hearts were used (n = 8–12 for each graph). *, significantly different from K⁺ alone by one-way ANOVA, followed by post hoc testing (Dunnett’s test).

Fig. 2. Carrier-mediated NE release from isolated ischemic mouse hearts. After a stabilization period, each of the 84 hearts was made globally ischemic by a 20-min perfusion with glucose-free KHS containing sodium dithionite 0.25 mM and bubbled with 95% N₂ plus 5% CO₂. Bars are means ± S.E.M. (n = 5–11 per column). Control NE release represents the total amount of NE released in the 20-min ischemic period. This release was carrier-mediated because it was inhibited by the NE transporter blocker desipramine (DMI). Drugs were administered at the following concentrations: 100 nM DMI, 100 nM imetit, 300 nM thioperamide, 100 nM CPA, and 100 nM DPCPX. The data show that when subjected to ischemia, H₃R⁻/⁻ hearts release a 2-fold greater amount of NE than do H₃R⁺/⁺ hearts (p < 0.01), despite the fact the adenosine-mediated modulatory system seems to be functioning as well in the H₃R⁻/⁻ as in the H₃R⁺/⁺ hearts. Note that the doubling of ischemic NE release in the H₃R⁻/⁻ hearts persists in the presence of imetit and thioperamide. *, significantly different from control by one-way ANOVA, followed by post hoc testing (Dunnett’s test).
tion. Also, because of ATP depletion and impaired NE storage in synaptic vesicles, NE accumulates in the axoplasm. These conditions force the reversal of the Na⁺-dependent NE transporter in an outward direction, triggering a massive carrier-mediated release of NE and arrhythmias (Lameris et al., 2000; Levi and Smith, 2000; Akiyama and Yamazaki, 2001). Indeed, NE overflow in myocardial ischemia directly correlates with the severity of arrhythmias (Imamura et al., 1996; Hatta et al., 1999; Maruyama et al., 1999).

We had identified H₃R as inhibitory heteroreceptors in adrenergic nerve endings of the heart (Endou et al., 1994). We also established that in addition to inhibiting NE exocytosis from sympathetic nerve endings, selective H₃R agonists attenuate carrier-mediated release of NE in both animal and human models of protracted myocardial ischemia (Imamura et al., 1996; Hatta et al., 1997). We subsequently demonstrated that H₃R-mediated attenuation of exocytotic NE release involves an inhibition of N-type Ca²⁺ channels (Silver et al., 2002), whereas H₃R-mediated reduction of carrier-mediated NE release is associated with diminished Na⁺/H⁺ exchanger activity (Imamura et al., 1996; Hatta et al., 1997; Silver et al., 2001). Most important, by reducing ischemic NE release, H₃R stimulation significantly attenuates the severity of ischemic arrhythmias (Imamura et al., 1996; Levi and Smith, 2000).

Other presynaptic receptors, such as α₁ adrenoceptors and α₂R, also modulate NE release from cSNE (Seyedi et al., 1997). Yet, H₃R stimulation attenuates both exocytotic and carrier-mediated NE release, whereas α₁-adrenoceptor agonists attenuate NE exocytosis but enhance carrier-mediated NE release (Imamura et al., 1996). Furthermore, although α₂R activation reduces both exocytotic and carrier-mediated NE release, α₂R stimulation has negative chronotropic and dromotropic effects, whereas H₃R agonists have no such effects (Levi and Smith, 2000). Accordingly, because excess NE release can trigger severe arrhythmias and sudden cardiac death, we have proposed that negative modulation of NE release by H₃R agonists may offer a novel therapeutic approach to myocardial ischemia (Levi and Smith, 2000; Mackins and Levi, 2000).

The recent creation of a transgenic line of mice devoid of H₃R (Toyota et al., 2002) provided us with the opportunity to assess the relevance of H₃R in myocardial ischemia. Thus, we found that although cSNE isolated from wild-type mice responded to the H₃R agonist imetit with a marked decrease in K⁺-induced NE release, similar to what we had observed previously in SNE isolated from guinea pig, dog, and human hearts (Endou et al., 1994; Imamura et al., 1994, 1995; Seyedi et al., 1996; Hatta et al., 1997), cSNE isolated from H₃R⁻/- mice failed to respond to H₃R agonists with an attenuation of NE exocytosis. Yet H₃R⁻/- cSNE still responded to A₁R agonists, as demonstrated by the fact that CPA attenuated equally effectively NE exocytosis in cSNE of H₃R⁻/⁻ and H₃R⁺/⁺ mice. These findings clearly indicate that H₃R⁻/- mice are an ideal model for the verification of the postulated cardioprotective role of H₃R located on cSNE.

Indeed, we found that in ischemic conditions, a lack of H₃R in cSNE translated into a 2-fold increase in NE overflow from the hearts of H₃R⁻/- mice compared with H₃R⁺/⁺ hearts. This is consistent with our previous findings in the guinea pig heart, in which the blockade of H₃R with thioperaamide doubled NE release during ischemia/reperfusion (Imamura et al., 1994), and in a human model of myocardial ischemia, in which blockade of H₃R with thioperaamide or clobenpropit significantly increased NE release (Hatta et al., 1997). The massive NE overflow from H₃R⁻/- mouse hearts occurred despite the fact that inhibitory A₁Rs were still functioning to attenuate both exocytotic and carrier-mediated NE release in the H₃R⁻/- hearts. This clearly demonstrates that cSNE H₃Rs play a relevant role in the modulation of NE release in myocardial ischemia.

Notably, the H₃R antagonist thioperaamide potentiated NE release from ischemic H₃R⁻/- hearts but not from cSNE from normoxic H₃R⁺/⁺ hearts. This indicates that, as we had observed previously in guinea pig and human hearts, H₃Rs located on cSNE become activated in conditions characterized by enhanced adrenergic activity, such as myocardial ischemia, when cSNE are exposed to functionally significant concentrations of histamine released from local mast cells by oxygen free radicals (Imamura et al., 1994; Hatta et al., 1997). The fact that thioperaamide failed to potentiate NE overflow from ischemic H₃R⁻/- hearts further strengthens this notion. Basal NE release from cSNE isolated from H₃R⁻/- was ~60% greater than that from cSNE isolated from H₃R⁺/⁺ hearts. This finding is consistent with a recent report of constitutive activity of native H₃R in rodent brain (Morisset et al., 2000).

Inasmuch as excessive NE release is recognized as a major cause of arrhythmic cardiac dysfunction in humans (Braunwald and Sobel, 1988; Dart and Du, 1993; Kubler and Strasser, 1994; Benedict et al., 1996), our present and past findings reveal that H₃R perform a crucial protective role in myocardial ischemia. This adds further strength to our notion (Levi and Smith, 2000; Mackins and Levi, 2000) that negative modulation of NE release by H₃R agonists may offer a novel therapeutic approach to myocardial ischemia.

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References


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