Histamine 3 Receptor Activation Reduces the Expression of Neuronal Angiotensin II Type 1 Receptors in the Heart

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ABSTRACT

In severe myocardial ischemia, histamine 3 (H3) receptor activation affords cardioprotection by preventing excessive norepinephrine release and arrhythmias; pivotal to this action is the inhibition of neuronal Na+/H+ exchanger (NHE). Conversely, angiotensin II, formed locally by mast cell-derived renin, stimulates NHE via angiotensin II type 1 (AT1) receptors, facilitating norepinephrine release and arrhythmias. Thus, ischemic dysfunction may depend on a balance between the NHE-modulating effects of H3 receptors and AT1 receptors. The purpose of this investigation was therefore to elucidate the H3/AT1 receptor interaction in myocardial ischemia/reperfusion. We found that H3 receptor blockade with clobenpropit increased norepinephrine overflow and arrhythmias in Langendorff-perfused guinea pig hearts subjected to ischemia/reperfusion. This coincided with increased neuronal AT1 receptor expression. NHE inhibition with cariporide prevented both increases in norepinephrine release and AT1 receptor expression. Moreover, norepinephrine release and AT1 receptor expression were increased by the nitric oxide (NO) synthase inhibitor L-nor-methyl-L-arginine and the protein kinase C activator phorbol myristate acetate. H3 receptor activation in differentiated sympathetic neuron-like PC12 cells permanently transfected with H3 receptor cDNA caused a decrease in protein kinase C activity and AT1 receptor protein abundance. Collectively, our findings suggest that neuronal H3 receptor activation inhibits NHE by diminishing protein kinase C activity. Reduced NHE activity sequentially causes intracellular acidification, increased NO synthesis, and diminished AT1 receptor expression. Thus, H3 receptor-mediated NHE inhibition in ischemia/reperfusion not only opposes the angiotensin II-induced stimulation of NHE in cardiac sympathetic neurons, but also down-regulates AT1 receptor expression. Cardioprotection ultimately results from the combined attenuation of angiotensin II and norepinephrine effects and alleviation of arrhythmias.

Introduction

In severe myocardial ischemia, norepinephrine (NE) is abundantly carried out of sympathetic nerve terminals by the NE transporter functioning in a reversed outward mode; this nonvesicular NE release process is known as “carrier-mediated” (Scho¨mig, 1990; Levi and Smith, 2000). Carrier-mediated NE release is a key arrhythmogenic determinant; pivotal for its occurrence is the activation of neuronal Na+/H+ exchanger (NHE) (Levi and Smith, 2000). Angiotensin II (ANG II), formed locally by mast cell-derived renin in myocardial ischemia (Mackins et al., 2006; Reid et al., 2007), is a major NHE activator via ANG II type 1 receptors (AT1Rs).

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ABBREVIATIONS: NE, norepinephrine; ANG, angiotensin; AT, angiotensin; AT1, AT1 receptor; AT2, AT1 receptor; AMD, ATP-methyl-L-arginine and the protein kinase C activator phorbol myristate acetate; H3, histamine 3 receptor; H4, histamine 4 receptor; I/R, ischemia/reperfusion; l-NMA, L-nor-methyl-L-arginine; NHE, Na+/H+ exchanger; NO, nitric oxide; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; TBS, Tris-buffered saline; NGF, nerve growth factor; ANOVA, analysis of variance; ACE2, angiotensin I converting enzyme 2; CBP, clobenpropit; EXP3174, 2-n-butyl-4-chloro-1-((2’-(1H-tetrazol-5-yl) biphenyl-4-yl) methyl) imidazole-5-carboxylic acid; HOE642, 4-isopropyl-3-methylsulfonylbenzoyl-guanidine methanesulfonate.

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(Koyama et al., 2003a,b). Thus, the magnitude of carrier-mediated NE release and associated arrhythmias may depend on a balance between the respective NHE-modulating effects of H₂R and AT₁R.

The purpose of this investigation was therefore to ascertain whether enhanced ischemic cardiac dysfunction, which is manifest when H₂Rs are blocked or deleted, results from an unimpeded AT₁R-NHE activation. We report that H₂R activation at the level of sympathetic nerve endings in myocardial ischemia opposes the deleterious effects of locally formed ANG II, not only by counteracting the NHE-stimulating effect of ANG II, but also by reducing the expression of AT₁R.

Materials and Methods

Perfusion of Guinea Pig Hearts Ex Vivo. All experiments were approved by the Institutional Animal Care and Use Committee of Weill Cornell Medical College. Male Hartley guinea pigs (Charles River Laboratories, Stone Ridge, NY), weighing 300 to 350 g, were anesthetized with CO₂ and euthanized by exsanguination while under anesthesia. Hearts were rapidly isolated and perfused at constant pressure (40 cm of H₂O) in a Langendorff apparatus with a modified Ringer's solution composed of 154 mM NaCl, 5.6 mM KCl, 2.2 mM CaCl₂, 5.6 mM glucose, and 6 mM NaHCO₃. The perfusion fluid was equilibrated with 100% O₂ at 37°C. After a 20-min stabilization period, normothermic ischemia was induced by complete cessation of coronary perfusion, followed by 30-min reperfusion. Hearts receiving drug treatment were treated for 15 min before the induction of ischemia. All drugs were added to the perfusion solution. The coronary effluent was collected into tubes. In the preischemic and ischemic periods, tubes were replaced every 2 min. The volume of effluent collected for each period was weighed and subsequently analyzed for NE content. NE was assayed in the coronary perfusate by high-pressure liquid chromatography with electro-chemical detection (Seyedi et al., 1999). Surface ECG was obtained from leads attached to the left ventricle and the right atrium and analyzed by using PowerLab/8SP (ADInstruments Inc., Colorado Springs, CO). Onset and duration of reperfusion arrhythmias were recorded and quantified according to the Lambeth Conventions (Walker et al., 1988).

Preparation of Cardiac Synaptosomes. Guinea pigs were anesthetized with CO₂ and exsanguinated while under anesthesia (see above). The rib cage was rapidly opened, and the heart was dissected away. A cannula was inserted in the aorta, and the heart was perfused at constant pressure (40 cm of H₂O) in a Langendorff apparatus with oxygenated Ringer's solution. Hearts receiving drug treatment were treated for 15 min before the induction of ischemia. After a 20-min stabilization period, normothermic ischemia was induced by complete cessation of coronary perfusion for 20 min. After a 20-min normothermic ischemia, hearts were subsequently freed from fat and connective tissue and minced in Tris-buffered saline (TBS; 0.28 M sucrose containing 50 mM Tris and 1 mM EDTA, pH 7.4) containing protease inhibitor cocktail (BioVision Research Products, Mountain View, CA). After low-speed centrifugation (10 min at 120g at 4°C), the supernatants were centrifuged for 20 min at 20,000g at 4°C. This pellet, which contained cardiac synaptosomes (pinched-off sympathetic nerve endings), was resuspended in the same Tris-buffered saline with 0.1% Triton X-100. The concentration of protein in homogenate was determined by using Bio-Rad protein assay solution (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin as a standard.

For Western blot, membrane proteins were loaded and run on standard 10 to 20% gradient NuPAGENovex Bis-Tris Mini Gel (Invitrogen, Carlsbad, CA) in NuPAGE MOPS Running Buffer (Invitrogen). Electrophoresis was carried out at 200 V and 100 mA for 70 min. Proteins were transferred onto polyvinylidene difluoride membranes (Immobilon-P; Millipore Corporation, Billerica, MA) for 90 min at 200 V and 300 mA at 4°C. The membranes were blocked in a blocking buffer (TBS containing 0.1% Tween 20 and 5% nonfat dry milk) at room temperature for 2 h. The membrane was then probed overnight at 4°C with rabbit anti-AT₁ receptor polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) (1:1000) in the antibody dilution buffer (TBS containing 0.1% Tween 20 and 5% bovine serum albumin). After the membrane was washed four times in TBS containing 0.1% Tween 1% were washed four times with TBS containing 0.1% Tween 20, and the bound antibodies were detected by using enhanced chemiluminescence (Millipore Corporation) followed by exposure to X-ray film (BioMax MR; Eastman Kodak, Rochester NY). Bands were analyzed by densitometry using Fluorchem8800 (Alpha Innotech, San Leandro CA), and the content of β-actin, which was detected by mouse monoclonal anti-human β-actin IgG-horseradish peroxidase conjugate (1:10,000; Alpha Diagnostic International, San Antonio, TX), was used as a control to ensure that the same amount of protein was loaded in each lane.

Cell Culture. PC12 cells, an AT₁R-expressing cell line derived from a pheochromocytoma of the rat adrenal medulla (Zhou et al., 2006), were transfected with the human H₂ receptor (donated by Dr. T. W. Lovenberg, Johnson and Johnson Pharmaceutical Research and Development, LLC) using Lipofectamine 2000 (Invitrogen) following the manufacturer's protocol (Morrey et al., 2008). PC12-H₂ cells line were selected and maintained in selection media containing 500 µg/ml G418 sulfate (Meditech, Herndon, VA) and/ or Zeocin (Invitrogen), respectively (Morrey et al., 2008).

To examine H₂R-mediated effects on AT₁R expression, PC12-H₂ cells were first grown on collagen from rat tail (Sigma-Aldrich, St. Louis, MO) and cultured in six-well plates in Dulbecco's modified Eagle's medium for 4 to 5 subsequent days containing 0.5% horse serum, 1% fetal bovine serum, G418 sulfate (Meditech), streptomycin (10 µg/ml), amphotericin B (250 ng/ml), penicillin (100 U), and 1 ng/ml nerve growth factor (NGF). Cells were exclusively used from passages 15 to 24 and grown at 37°C in a humidified atmosphere of 95% air/5% CO₂. To determine H₂R effects, cells were stimulated with imetit [S-(2-(4-imidazolyl))ethyl]isothiourea] (100 µM) for 30 min. In further experiments, cells were additionally treated with the H₂R antagonist clobenpropit [S-(3-(4(5)-imidazolyl))propyl-N4-chlorobenzyl]isothiourea] (50 nM). Vehicle-treated cells served as control. Cell lysis buffer (Cell Signaling Technology) was added to the cells, and the expression of AT₁R and protein kinase C (PKC) activity in the lysates were examined by Western blotting.

PKC Assay. PC12-H₂ cells were incubated with phorbol 12-myristate β-acetate (PMA) (30 min) with or without imetit pretreatment (10 min). Other cells were pretreated with clobenpropit (10 min) before imetit and PMA treatment. Cells were then homogenized in buffer (200 µl) (20 mM Tris-HCl, 2 mM EDTA, 10 mM EGTA, 0.25 M sucrose, β-mercaptoethanol, and 1× protease inhibitors cocktail). Cell homogenates were then passed through syringes with a needle (30 gauge) 10 times, and cell lysates were centrifuged at 100,000g for 30 min to collect cytosolic fractions (supernatant). The pellets were resuspended in homogenization buffer (50 µl) with 1% Triton X-100, and then centrifuged at 100,000g for 30 min to collect membrane fractions (supernatant). Translocation of PKC was assessed by using a PKC-specific antibody (Santa Cruz Biotechnology, Inc.; 1:1000 dilution) in Western blot analysis. Methods for Western blot analysis were as described previously (Corti et al., 2011). The ratio of PKC in membrane to that in cytosol was expressed as PKC translocation (i.e., PKC activity).

Drugs. Imetit was purchased from Tocris Bioscience (Ellisville, MO). Clobenpropit and N⁶-methyl-l-arginine (l-NMA) were purchased from Sigma-Aldrich (St. Louis, MO). EXP3174 [2-n-butyl-4-
H₂R-Mediated Cardioprotective Effects Involve NHE Attenuation and AT₁R Inhibition. Langendorff-perfused guinea pig hearts were subjected to global ischemia for 20 min, followed by reperfusion for 30 min (Imamura et al., 1999). Reperfusion was characterized by an increase in NE overflow into the coronary effluent (~140 pmol/g from an undetectable level in preischemic conditions) (Fig. 1). In hearts perfused with the H₂R antagonist clobenpropit (50 nM) NE overflow was ~2.5-fold greater than in I/R control hearts (Fig. 1). The NHE inhibitor HOE642 (cariporide; 10 μM) reduced the clobenpropit-induced increase in NE overflow by ~40% (Fig. 1), whereas the AT₁R antagonist EXP3174 (100 nM) abolished it (Fig. 1). These results suggested that H₂R blockade uncovers a protective H₂R-mediated effect in I/R characterized by decreased NE release, an effect probably involving NHE attenuation and AT₁R inhibition.

The enhanced NE spillover in I/R hearts was accompanied by long-lasting reperfusion arrhythmias (i.e., ventricular tachycardia and ventricular fibrillation) whose duration was greatly increased in hearts perfused with the H₂R antagonist clobenpropit (50 nM). In contrast, in the presence of the NHE inhibitor cariporide (10 μM) ventricular tachycardia/ventricular fibrillation duration was shortened and abolished in the presence of the AT₁R antagonist EXP3174 (100 nM) (data not shown). Thus, the H₂R-mediated NHE attenuation and AT₁R inhibition in I/R includes both a decrease in NE release and an alleviation of reperfusion arrhythmias.

**Results**

H₂R Activation during I/R Decreases AT₁R Expression in Cardiac Sympathetic Nerve Terminals: NHE Involvement. We next questioned whether this H₂R-mediated inhibition of AT₁R activity might entail a decrease in neuronal AT₁R expression. For this, we determined AT₁R expression in sympathetic nerve endings (synaptosomes) isolated from guinea pig hearts subjected to I/R. Langendorff-perfused guinea pig hearts were subjected to 20-min global ischemia, followed by 4-min reperfusion. Cardiac synaptosomes were isolated at the end of reperfusion, and expression of AT₁R was evaluated by Western blotting. AT₁Rs were detected as membrane proteins of 43-kDa molecular mass (Fig. 2, top). In the presence of the H₂R antagonist clobenpropit (50 nM) AT₁R expression increased by ~70%; this effect was prevented by NHE inhibition with cariporide (10 μM) and diminished by AT₁R blockade with EXP3174 (100 nM) (Fig. 2, bottom). These results suggested that constitutive activation of H₂R during I/R decreases the expression of AT₁R in cardiac sympathetic nerve terminals and that this effect is likely to involve NHE. Hence, we next investigated the mechanisms mediating the H₂R-induced attenuation of AT₁R expression.

Nitric Oxide Attenuates AT₁R Expression in Cardiac Sympathetic Nerve Terminals during I/R. By inhibiting NHE, H₂R activation can lead to intracellular acidification (Silver et al., 2001). In turn, intracellular acidification is known to stimulate nitric oxide (NO) production (Tsutsumi et al., 1999), and NO has been shown to suppress AT₁R expression (Ichiki et al., 1998). Hence, we questioned whether NO might be involved in the H₂R-induced reduction of AT₁R expression. For this, we perfused isolated guinea pig hearts with the NO synthase inhibitor L-NMA (100 μM) and then subjected them to 20-min global ischemia, followed by 4-min reperfusion. At the end of reperfusion, we isolated cardiac synaptosomes and measured the expression of AT₁R by Western blotting. The level of AT₁R expression in synapto-
somes isolated from hearts subjected to I/R in the presence of L-NMA was twice as large as in its absence (Fig. 3), and NE overflow was ~3-fold greater (Fig. 4). These findings implied that H3R activation during I/R leads to a NO-mediated attenuation of AT1R expression, resulting in a decreased NE release.

An H3R-Mediated PKC/NHE Attenuation Decreases AT1R Expression in Cardiac Sympathetic Nerve Terminals during I/R. Our findings suggested that NHE is probably involved in the H3R-induced attenuation of AT1R expression in sympathetic nerve terminals isolated from hearts subjected to I/R (see Fig. 2). PKC is known to directly phosphorylate and activate NHE (Wakabayashi et al., 1997; Karmazyn et al., 1999), whereas H3R activation attenuates NHE activity (Silver et al., 2001), possibly by decreasing phosphoinositide turnover (Cherifi et al., 1992), thus inhibiting PKC activation. Hence, we hypothesized that a decrease in PKC activity may play a role in the H3R-induced attenuation of AT1R expression. To verify this hypothesis, we subjected Langendorff-perfused guinea pig hearts to I/R in the presence of the PKC activator PMA (300 nM) and determined NE overflow and AT1R expression in sympathetic nerve endings after 4-min reperfusion. In synaptosomes isolated from I/R hearts perfused with PMA the level of AT1R expression was ~2.6-fold greater than in the absence of PMA (Fig. 3), whereas NE overflow was ~3.5-fold greater in the presence than in the absence of PMA (Fig. 4). These data suggested that constitutional H3R activation during I/R exerts cardioprotective effects that may result from a sequential decrease in PKC and NHE activity.

H3R Activation Induces a Decrease in AT1R Protein Expression and PKC Activity in PC12-H3 Cells. We directly tested the hypothesis that H3R activation decreases neuronal AT1R expression in a cell line bearing a sympathetic neuron phenotype, i.e., NGF-differentiated rat pheochromocytoma PC12 cells permanently transfected with H3R cDNA (PC12-H3 cells) (Morrey et al., 2008). In the presence of the H3R agonist imetit (100 nM), AT1R expression was decreased by ~40%; this effect was prevented by the H3R antagonist clobenpropit (50 nM). In the absence of H3R activation, clobenpropit had no effect on AT1R expression (Fig. 5).

We next directly tested the postulate that H3R activation decreases neuronal PKC activity. As evidence of PKC activation, we measured cytosol-to-membrane translocation of PKC in NGF-differentiated PC12-H3 cells. We found that H3R activation with imetit (100 nM) attenuated the increase in PKC translocation induced by PMA (300 nM), an effect that was prevented by the H3R antagonist clobenpropit (50 nM) (Fig. 6). These results suggested that H3R activation induces a decrease in neuronal AT1R protein expression that involves a reduction in PKC activity.

Discussion

The goal of our study was to determine whether H3Rs afford cardioprotection in I/R by counteracting the AT1R-mediated stimulation of NHE. We found that activation of neuronal H3R opposes the deleterious effects of locally formed ANG II, not only by inhibiting NHE, but also by...
reducing the expression of AT_1 R. This novel phenomenon broadens the known cardioprotective effects of H_3 R activation (Levi and Smith, 2000; Mackins and Levi, 2000). We had reported previously that H_3 Rs are activated in I/R and limit the release of pathological amounts of NE and associated reperfusion arrhythmias by decreasing NHE activity, thus counteracting a pivotal mechanism of nonvesicular, carrier-mediated NE release (Levi and Smith, 2000). Because ANG II is formed locally in the heart during I/R (Mackins et al., 2006) and ANG II is a major H_3 R activator (Reid et al., 2004), we assumed that in I/R the activation of H_3 R could counterbalance the NHE-stimulating effect of ANG II. Indeed, we found that pharmacological blockade of H_3 R in I/R enhanced the magnitude of NE overflow and the severity of arrhythmias, and this effect was prevented by NHE and AT_1 R blockade. This clearly indicated that in protracted I/R H_3 R activation serves to counteract the NHE-stimulating effect of intraneuronal anaerobic acidification and the AT_1 R-mediated effects of ANG II.

The first hint that H_3 R might do more than just antagonize ANG II at the NHE level came from the finding that AT_1 R protein abundance in synaptosomes isolated from I/R hearts increased when H_3 Rs were blocked. This, in fact, suggested that H_3 Rs may influence neuronal AT_1 R expression in hearts subjected to I/R. Inasmuch as cardiac synaptosomes probably include cholinergic, purinergic, and sensory C-fiber terminals (Seyedi et al., 1999; Morrey et al., 2010), we tested whether H_3 R activation would influence AT_1 R expression in PC12-H_3 cells, which once differentiated with NGF express a typical sympathetic neuron phenotype (Taupenot, 2007; Morrey et al., 2008; Corti et al., 2011). Indeed, we found that activation of H_3 R with imetit markedly diminished AT_1 R protein abundance in these cells, an action that was abolished by the H_3 R antagonist clobenpropit. It is noteworthy that, although the down-regulation of AT_1 R protein expression by H_3 R activation seemed to be fairly rapid, the time of exposure to the H_3 R agonist (i.e., 30 min in PC12-H_3 cells) was comparable with that reported for the down-regulation of AT_1 R by dopamine D_1 receptor activation in renal proximal tubule cells (i.e., half-life 0.47 ± 0.18 h) (Gildea et al., 2008).

It is noteworthy that the CBP-induced increase in NE overflow was completely suppressed by the AT_1 R blocker EXP3174, but partially suppressed by the NHE blocker cariporide, whereas cariporide completely blocked the CBP-induced AT_1 R up-regulation. These apparent discrepancies probably result from the fact that ANG II elicits NE release by two mechanisms, exocytotic and carrier-mediated (Reid et al., 2004), which are both mediated by AT_1 R, but are Ca_2+- and NHE-dependent, respectively. Accordingly, AT_1 R blockade by EXP3174 is likely to be more effective in preventing NE release because it blocks both exocytosis and carrier-mediated release. In contrast, cariporide only blocks NHE without affecting exocytosis (Levi and Smith, 2000). On the other hand, cariporide completely blocks the CBP-induced increase in AT_1 R expression because this H_3 R-mediated phenomenon seems to be solely NHE-dependent.

The finding that NHE blockade in I/R prevented the increase in AT_1 R expression in cardiac synaptosomes, whereas PKC stimulation with PMA increased it, implied an involvement of both NHE and PKC. First, we tested whether PKC could be involved in the H_3 R-mediated inhibition of NHE and associated reduction of AT_1 R expression. Given that PKC is translocated/activated in I/R (Strasser et al., 1992) and this stimulates NHE (Karmazyn et al., 1999), we thought it plausible that the H_3 R-induced inhibition of NHE may involve a decrease in PKC translocation/activation. Indeed, we found that the PKC-induced activation of AT_1 R was abolished by H_3 R activation. It is conceivable that this H_3 R-mediated decrease in PKC activation stems from a decreased phosphoinositide turnover, because H_3 Rs have been found previously to be negatively coupled to it (Cherifi et al., 1992). Although we did not examine which PKC isoform may be specifically involved in the down-regulation of AT_1 R in sympathetic nerve endings, if PKCε were involved (Koda et al., 2010) it could be plausibly activated by NO via a positive feedback mechanism (Ping et al., 1999). If so, this could counteract in part the H_3 R-mediated decrease in PKC activity.

An H_3 R-mediated decrease in NHE activity is expected to lead to acidification of cardiac sympathetic nerve endings (Levi and Smith, 2000), and intracellular acidification was shown to stimulate NO production (Tsutsuami et al., 1999). Moreover, NO production increases during I/R in the mammalian heart (Zweier et al., 1995a; Lecour et al., 2001), and NO has been shown to suppress AT_1 R expression (Ichiki et al., 1998). It thus became apparent that NO might mediate the decreased AT_1 R protein abundance in sympathetic nerve endings initiated by the H_3 R-mediated decrease in NHE activity. The finding that inhibition of NO synthase en-

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**Fig. 6. H_3 R activation attenuates the PKC-induced PKC activation (i.e., translocation from cytosol to membrane) in PC12-H_3 cells. Top, representative blots. Cells were treated with the PKC activator PMA (300 nM; 20 min), the H_3 R agonist imetit (100 nM; 10 min), and the H_3 R antagonist CBP (50 nM; 10 min). PKC expression in cytosolic (C) and membrane (M) fractions was determined by Western immunoblotting. Bottom, PKC translocation expressed as the ratio of PKC abundance between membrane and cytosolic fractions. Bars are means (± S.E.M.; n = 3) normalized to β-actin. #, P < 0.01 from PMA by unpaired t test; †, P < 0.05 from PMA + imetit + CBP by ANOVA and Bonferroni’s multiple test; ##, P < 0.05 from control by ANOVA and Bonferroni.**
hanced AT₁R expression in I/R synaptosomes, and markedly increased NE release, suggests that H₃R activation increases NO production, ultimately reducing AT₁R protein abundance in sympathetic nerve endings. Although we did not measure NO synthesis, our results with t-NAME indicate an involvement of NO and NO synthase. Yet, it is possible that NO was also produced by an enzyme-independent mechanism (e.g., by direct reduction of nitrite to NO) as demonstrated by Zweier et al. (1995b).

It is conceivable that additional NO might be generated by the activation of Mas, an orphan G protein-coupled receptor that is a functional ligand site for angiotensin 1 to 7 (ANG-1–7) (Santos et al., 2003). ANG-1–7 are produced most efficiently from ANG II by ACE2, a newly discovered carboxypeptidase (Raizada and Ferreira, 2007; Ferrario, 2010). Yet, although ACE2 is widely distributed throughout the brain (Dookey et al., 2007), its presence in peripheral sympathetic nerves has yet to be demonstrated. Thus, the contribution of ACE2 and ANG-1–7 to the formation of NO in cardiac sympathetic nerves and their possible roles in the down-regulation of AT₁R remain to be determined.

Although imidet and clobenpropit have long been considered to be a selective H₄R agonist and antagonist, respectively (Garbarg et al., 1992, Koyama et al., 2003b), they have also been shown to activate H₂R (Lim et al., 2005). Similar to H₂Rs, H₃Rs are G₂α-coupled; hence, it is conceivable that H₃Rs may share some of the characteristic effects of H₂Rs, such as NHE inhibition and thus, might contribute to the H₂R-mediated attenuation of AT₁R expression. Yet, although H₂Rs have been described in the central nervous system (Connelly et al., 2009), their presence in cardiac sympathetic neurons has not been reported. Thus, whether the H₂R agonistic effects of clobenpropit might have contributed to the increase in synaptosomal AT₁R expression (see Fig. 2) cannot be confirmed at this time.

In conclusion, we have uncovered a novel cardioprotective action resulting from activation of neuronal H₃R in mammalian heart (see Fig. 7). Binding of an endogenous ligand to H₃R, most likely histamine, released from local mast cells (Imamura et al., 1994; Hatta et al., 1997) by the action of reactive oxygen species produced during I/R (Koda et al., 2010), probably causes a decrease in phosphoinositide turnover (Cherifi et al., 1992). This is expected to reduce the formation of diacylglycerol and thus diminish PKC activity, as we have demonstrated here (see Fig. 6). This, in turn, decreases NHE activity (Karmazyn et al., 1999), so that H⁺ ions accumulate intracellularly. Intracellular acidification, as it occurs in myocardial ischemia (Poole-Wilson, 1989; Levi and Smith, 2000), stimulates the production of NO (Tetsu et al., 1999), which has been shown to suppress AT₁R expression (Ichiki et al., 1998). Hence, the H₃R-induced decrease in NHE activity leading to an increased NO synthesis may well be responsible for the ultimate decrease in AT₁R protein abundance (Fig. 7). We propose that our findings of a down-regulation of AT₁R signaling and attenuation of NE release by activation of neuronal H₃R are both crucial and plausibly translatable mechanisms of cardioprotection, not only in myocardial ischemia but also in other cardiac dysfunctions in which ANG II plays a major role, such as heart failure.

Acknowledgments

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Performed data analysis: Hashikawa-Hobara and Chan.
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References

Koyama M, Heerdt PM, and Levi R (2003a) Increased severity of reperfusion ar-

Fig. 7. Proposed mechanisms for the H₃R-induced reduction of neuronal AT₁R expression. DAG, diacylglycerol; PI, phosphoinositide; PIP₂, phosphatidylinositol-4,5-biphosphate; PLC, phospholipase C.


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