Natriuretic Peptide-Induced Catecholamine Release from Cardiac Sympathetic Neurons: Inhibition by Histamine H$_3$ and H$_4$ Receptor Activation

Noel Yan-Ki Chan, Pablo A. Robador, and Roberto Levi

Department of Pharmacology, Weill Cornell Medical College, New York, New York

ABSTRACT

We reported previously that natriuretic peptides, including brain natriuretic peptide (BNP), promote norepinephrine release from cardiac sympathetic nerves and dopamine release from differentiated pheochromocytoma PC12 cells. These proexocytotic effects are mediated by an increase in intracellular calcium secondary to cAMP/protein kinase A (PKA) activation caused by a protein kinase G (PKG)-mediated inhibition of phosphodiesterase type 3 (PDE3). The purpose of the present study was to search for novel means to prevent the proadrenergic effects of natriuretic peptides. For this, we focused our attention on neuronal inhibitory G$\alpha_{i/o}$-coupled histamine H$_3$ and H$_4$ receptors. Our findings show that activation of neuronal H$_3$ and H$_4$ receptors inhibits the release of catecholamines elicited by BNP in cardiac synaptosomes and differentiated PC12 cells. This effect results from a decrease in intracellular Ca^{2+} due to reduced intracellular cAMP/PKA activity, caused by H$_3$ and H$_4$ receptor-mediated PKG inhibition and consequent PDE3-induced increase in cAMP metabolism. Indeed, selective H$_3$ and H$_4$ receptor agonists each synergized with a PKG inhibitor and a PDE3 activator in attenuating BNP-induced norepinephrine release from cardiac sympathetic nerve endings. This indicates that PKG inhibition and PDE3 stimulation are pivotal for the H$_3$ and H$_4$ receptor-mediated attenuation of BNP-induced catecholamine release. Cardiac sympathetic overstimulation is characteristic of advanced heart failure, which was recently found not to be improved by the administration of recombinant BNP (nesiritide), despite the predicated beneficial effects of natriuretic peptides. Because excessive catecholamine release is likely to offset the desirable effects of natriuretic peptides, our findings suggest novel means to alleviate their adverse effects and improve their therapeutic potential.

Introduction

Although natriuretic peptides have been viewed as a compensatory neurohormonal system that is up-regulated in the setting of heart failure, affording beneficial cardiac and hemodynamic effects via particulate guanylyl cyclase stimulation and increased cGMP formation (Molkentin, 2003; Munagala et al., 2004), their role in alleviating cardiac ailments has been challenged (Wang et al., 2004; Simon et al., 2008). Indeed, in a recent large clinical trial, the administration of nesiritide [recombinant brain natriuretic peptide (BNP)] was found not to protect patients with acute heart failure (O'Connor et al., 2011).

We had reported previously that BNP promotes norepinephrine (NE) release in the guinea pig heart ex vivo, an effect that is further enhanced in ischemia/reperfusion (Chan et al., 2012). We also found that natriuretic peptides, sodium nitroprusside, and cell-permeable cGMP analogs all elicit catecholamine exocytosis in sympathetic nerves isolated from the guinea pig heart (i.e., cardiac synaptosomes) and in nerve growth factor (NGF)-differentiated PC12 cells, which bear a sympathetic nerve-ending phenotype (Chan et al., 2012). This proexocytotic effect results from an increase in intracellular calcium (Ca^{2+}). The process involves a protein kinase G (PKG)-mediated inhibition of phosphodiesterase type 3 (PDE3), which increases cAMP and protein kinase A (PKA) activity (Chan et al., 2012).

ABBREVIATIONS: BNP, brain natriuretic peptide; ANOVA, analysis of variance; DA, dopamine; 8-Br-cGMP, 8-bromoguanosine-3',5'-cyclic monophosphate; H$_3$R, H$_3$ receptor; H$_4$R, H$_4$ receptor; HBS, HEPES-buffered saline solution; NE, norepinephrine; NGF, nerve growth factor; PDE3, phosphodiesterase type 3; PKA, protein kinase A; PKG, protein kinase G; Rp-8-Br-cGMPS, 8-bromoguanosine-3',5'-cyclic monophosphorothioate; VASP, vasodilator-stimulated phosphoprotein; JNJ5207852, 1-{3-[4-(piperidin-1-ylmethyl)phenoxy]propyl}piperidine; A943931, 4-((3R)-3-amino-pyrrolidin-1-yl)-6,7-dihydro-5H-benzo[6,7]cyclohepta[1,2-d]pyrimidin-2-ylamine.

More recently, it was reported that BNP increases heart rate in mice by activating the guanylyl cyclase-linked natriuretic peptide A and B receptors and inhibiting PDE3 activity, resulting in an increase in L-type Ca^{2+} current (Springer et al., 2012). An association of BNP with cardiac sympathetic overdrive, originating from altered Ca^{2+} handling and culminating in ventricular arrhythmia, was also recently described in mice (Thireau et al., 2012).

Thus, it is conceivable that the proadrenergic effects of natriuretic peptides may offset their beneficial hemodynamic effects, as implied by the findings that β-adrenoceptor blockade protects the heart from the deleterious effects of BNP (Fujimura et al., 2009; Thireau et al., 2012). Given that an enhanced NE release bears dysfunctional and arrhythmogenic consequences (Schömig, 1990; Meredith et al., 1991; Levi and Smith, 2000; Grassi et al., 2009), we investigated novel means to reduce the NE-releasing effect of natriuretic peptides, hoping that they might eventually enable a safe and effective treatment of congestive heart failure with natriuretic peptides. For this, we focused our attention on neuronal histamine H$_3$ receptors, which are Gα$_{i/o}$-coupled and effectively inhibit physiologic and pathophysiologic NE release (Imamura et al., 1995; Seyedi et al., 1997; Levi and Smith, 2000). Likewise, histamine H$_4$ receptors are also Gα$_{i/o}$-coupled (Nijmeijer et al., 2012) and seem to be present in central and peripheral neurons (Nakaya et al., 2004; Connelly et al., 2009). Therefore, we ascertained the presence of H$_4$ receptors in cardiac sympathetic nerve terminals and investigated their possible modulation of BNP-induced NE release.

We report that the activation of neuronal H$_3$ and H$_4$ receptors inhibits the release of catecholamines elicited by BNP, and this effect results from a decrease in intracellular Ca^{2+}. This process involves a decrease in intracellular cAMP and PKA activity, based on H$_3$ and H$_4$ receptor-mediated PKG inhibition and consequent PDE3-induced increase in cAMP metabolism.

Materials and Methods

NE Release from Cardiac Synaptosomes. Male Hartley guinea pigs weighing 300 to 350 g (Charles River Laboratories, Inc., Wilmington, MA) 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. Spontaneously beating hearts were perfused through the aorta for 15 min at constant pressure (40 cm of H$_2$O) with Ringer's solution at 37°C saturated with 5% CO$_2$ and 95% O$_2$. Ringer's solution composition was 154 mM NaCl, 5.6 mM KCl, 2.2 mM CaCl$_2$, 6.0 mM NaHCO$_3$, and 5.6 mM dextrose. This procedure ensured that no blood traces remained in the coronary circulation. At the end of the perfusion, the hearts were minced in ice-cold HEPES-buffered saline solution (HBS), which contained 50 mM HEPES, pH 7.4, 144 mM NaCl, 5 mM KCl, 1.2 mM CaCl$_2$, 1.2 mM MgCl$_2$, and 10 mM glucose. Synaptosomes were isolated as described previously (Seyedi et al., 1997). Minced tissue was digested with 40 mg of collagenase (type II; Worthington Biochemicals, Freehold, NJ) per 10 ml of HBS per gram of wet heart weight for 1 h at 37°C. HBS contained 1 mM pargyline to prevent enzymatic destruction of NE. After low-speed centrifugation (10 min at 120g and 4°C), the resulting pellet was suspended in 10 volumes of 0.32 M sucrose and homogenized with a Teflon/glass homogenizer. The homogenate was spun at 650g for 10 min at 4°C, and the pellet was then rehomog-

enized and respun. The pellet containing cellular debris was discarded, and the supernatants from the last two spins were combined and equally subdivided into tubes. Each tube was centrifuged for 20 min at 20,000g at 4°C. This pellet, which contained cardiac synaptosomes, was resuspended in HBS to a final volume of 1 ml in a water bath at 37°C. Each suspension functioned as an independent sample and was used only once. In every experiment, one sample was untreated (control, basal NE release), and others were incubated with BNP for 10 min. When drugs were used, synaptosomes were preincubated with drugs for 10 min. When antagonists were used, samples were incubated with the antagonists before incubation with the agonist. Controls were incubated for an equivalent length of time without drugs. At the end of the incubation period, each sample was centrifuged (20 min, 20,000g, 4°C). The supernatant was assayed for NE content by high-pressure liquid chromatography with electrochemical detection (Seyedi et al., 1997). The pellet was assayed for protein content by a modified Lowry procedure (Seyedi et al., 1997).

Cell Culture. Rat pheochromocytoma PC12 cells were transfected with the human histamine H$_3$ receptor (donated by Dr. T. W. Lovenberg, Johnson and Johnson Pharmaceutical R&D, LLC, San Diego, CA) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. PC12-H$_3$ cell lines were selected and maintained in selection media containing 500 μg/ml G-418 sulfate (Mediatech, Herndon, VA). PC12 and PC12-H$_3$ cells were maintained in Dulbecco's modified Eagle's medium plus 10% fetal bovine serum, 5% donor horse serum, 1% L-glutamine, and antibiotics at 37°C in 5% CO$_2$. The differentiating protocol involved plating PC12 and PC12-H$_3$ cells on tissue culture plates coated with collagen (rat tail type VII; Sigma-Aldrich, St. Louis, MO) combined with exposure to low serum medium containing 1% fetal bovine serum, 0.5% donor horse serum, 1% L-glutamine, and antibiotics supplemented with 7S-NGF (BD Biosciences Discovery Labware, Bedford, MA). For each experiment, the culture medium was aspirated and cells were washed twice with Na-Ringer's (140 mM NaCl, 5 mM KCl, 10 mM HEPES, 1 mM MgCl$_2$, 2 mM glucose, and 2 mM CaCl$_2$), then incubated with BNP (100 nM), for 20 min in an incubator at 37°C either in the absence or presence of methimepip (histamine H$_3$ receptor agonist; 1 nM) (Kitbunnadaj et al., 2005), 4-methylhistamine (histamine H$_4$ receptor agonist; 20 μM) (Lim et al., 2005), 1-{3-[4-(piperidin-1-ylmethyl)phenoxy]propyl}piperidine (JNJ5207852) (histamine H$_3$ receptor antagonist; 30 nM) (Barbier et al., 2004), or 4-((3R)-3-amino-pyrrolidin-1-yl)-6,7-dihydro-5H-benzo[6,7]cyclohepta[1,2-d]pyrimidin-2-ylamine (A943931) (histamine H$_4$ receptor antagonist; 300 nM) (Cowart et al., 2008). When these drugs were used, PC12-H$_3$ cells were preincubated with them for 10 min. Controls were incubated for an equivalent length of time without drugs. At the end of each experiment aliquots of the supernatant and cell lysates (after a 30-min treatment with Triton X-100) were taken from each well and analyzed for dopamine (DA) content by high-performance liquid chromatography with electrochemical detection with a 6-min retention time. Other cell lysates were analyzed for histamine H$_3$ and H$_4$ receptor expression by Western blotting, intracellular cAMP levels, PKA activity, intracellular Ca^{2+}, PKG activity, or PDE3 activity.

Intracellular Ca^{2+} Assay. Cells were washed twice with Na-Ringer's, and then treated with potassium (100 mM; 3 min) or BNP (100 nM; 10 min) in the presence or absence of methimepip (histamine H$_3$ receptor agonist; 1 nM), 4-methylhistamine (histamine H$_4$ receptor agonist; 20 μM), JNJ5207852 (histamine H$_3$ receptor antagonist; 30 nM), or A943931 (histamine H$_4$ receptor antagonist; 300 nM). Controls were incubated for an equivalent length of time without drugs. At the end of each experiment, cells were washed with Dulbecco's phosphate-buffered saline containing 10 mM EGTA (to chelate external Ca^{2+}) and then with normal phosphate-buffered saline to remove the remaining EGTA. Cells were then lysed with the addition of water and harvested with a scraper. Ca^{2+} content was determined by using a Ca^{2+} assay kit (QuantiChrom Ca^{2+} Assay Kit; BioAssay Systems, Hayward, CA). The Ca^{2+} content was adjusted by

the protein content of the cells and expressed as milligrams of Ca^{2+} per milligram of protein.

cAMP Assay. Cells were treated and lysed as described above. Intracellular cAMP levels were determined by using a cAMP Biotrak EIA kit (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK) following the manufacturer's protocol. This cAMP assay is highly specific and based on competition between unlabeled cAMP and a fixed quantity of peroxidase-labeled cAMP for a limited number of binding sites on a cAMP-specific antibody. The cross-reactivity for cGMP, AMP, ADP, and ATP is below 0.01%, whereas cAMP is 100%.

PKA Activity. PKA phosphorylation (an indication of PKA activation) was measured by using a phosphorylated-PKAα antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) in Western blot. Methods for Western blot analysis were as described previously (Chan et al., 2012).

PKG Activity. Phosphorylated vasodilator-stimulated phosphoprotein (VASP; a major substrate for PKG) at Ser239 is a sensitive biochemical marker for monitoring the activity of PKG (Gill et al., 2007). VASP phosphorylation (i.e., PKG activity) was measured by using a phosphorylated-VASP antibody (Santa Cruz Biotechnology, Inc.) in Western blot. Methods for Western blot analysis were as described previously (Chan et al., 2012).

PDE3 Activity. PDE3 activity was measured by using a commercially available colorimetric PDE assay kit (Enzo Life Sciences, Farmingdale, NY) as described previously (Chan et al., 2012). Cell lysates were prepared and then total protein concentration was measured as described above. Free phosphate contamination was removed according to the manufacturer's protocol. Samples were incubated for 10 min at 37°C, and reactions were stopped with Biomol Green (Enzo). Samples were then put on a shaker for 20 min at room temperature. Results were measured by using a microplate reader (Molecular Devices, Sunnyvale, CA). PDE3-specific cAMP-hydrolytic activity was expressed as the difference between cAMP hydrolyzed (expressed as nmol/min/mg protein) in the presence and absence of the specific PDE3 inhibitor cilostamide.

Drugs and Chemicals. BNP was purchased from AnaSpec, Inc. (Fremont, CA); 8-Br-cGMP, forskolin, Rp-8-Br-cGMPS, insulin, and cilostamide were purchased from Sigma-Aldrich. Methimepip, JNJ5207852, 4-methyl histamine, and A943931 were purchased from Tocris Bioscience (Ellisville, MO).

Statistics. Data are presented as mean ± S.E.M. Parametric tests were used throughout the study. Either unpaired t test or one-way ANOVA followed by post hoc Dunnett's test was used in all figures. GraphPad Prism version 4.03 for Windows (GraphPad Software, Inc., San Diego, CA) was used. Values of $P < 0.05$ were considered statistically significant.

Results

K⁺- and BNP-Induced Norepinephrine Release from Cardiac Sympathetic Nerve Endings: Attenuation by Histamine H₃ and H₄ Receptor Activation.

Depolarization of isolated cardiac synaptosomes with extracellular potassium (100 mM) elicited a ~25% increase in NE release (Fig. 1, A and B). In the presence of the histamine H₃ receptor agonist methimepip (1 nM) (Kitbunnadaj et al., 2005) the K⁺-induced increase in NE release was reduced by ~50%, an effect that was abolished by the selective H₃ receptor antagonist JNJ5207852 (30 nM) (Barbier et al., 2004) (Fig. 1A). The K⁺-induced increase in NE release was also attenuated by ~54% by the selective H₄ receptor agonist 4-methylhistamine (20 μM) (Lim et al., 2005) (Fig. 1B). This effect was abolished by the selective H₄ receptor antagonist A943931 (300 nM) (Cowart et al., 2008) (Fig. 1B).
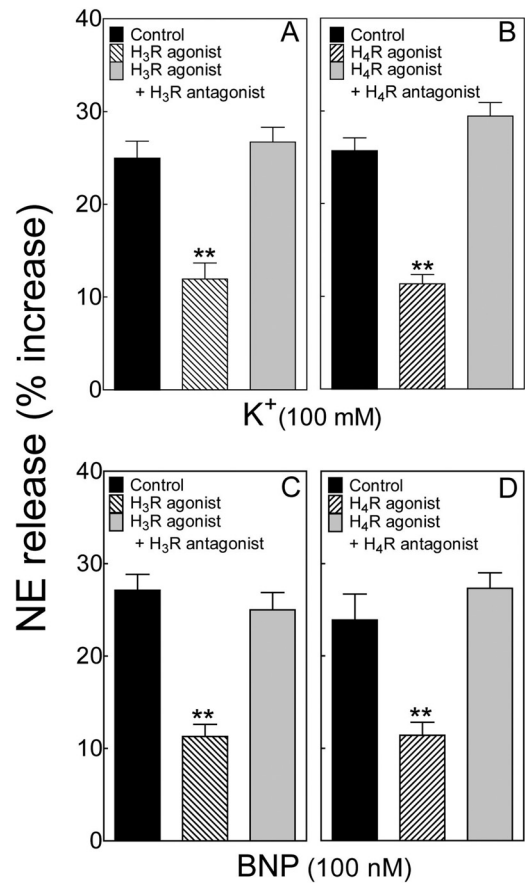
Incubation of isolated cardiac synaptosomes with BNP

Fig. 1. Activation of histamine H₃ and H₄ receptors attenuates K⁺- and BNP-induced NE release from cardiac sympathetic nerve endings. A and B, release of endogenous NE from guinea pig heart synaptosomes by depolarization with K⁺ (100 mM) in the absence and presence of the selective H₃ receptor agonist methimepip (1 nM) or the selective H₄ receptor agonist 4-methylhistamine (20 μM), respectively. Each agonist was used either alone or together with the respective selective antagonist, JNJ5207852 (H₃ receptor antagonist; 30 nM) and A943931 (H₄ receptor antagonist; 300 nM). Bars represent mean increases in NE release above basal level (± S.E.M.; $n = 8$ and 12 for A and B, respectively). Basal NE level was 255.4 ± 16.8 pmol/mg of protein ($n = 36$). ∗∗, $P < 0.01$ from corresponding control by one-way ANOVA followed by post hoc Dunnett's test. C and D, release of endogenous NE from guinea pig heart synaptosomes by human BNP (100 nM; 10-min exposure) in the absence and presence of the selective H₃ receptor agonist methimepip (1 nM) or the selective H₄ receptor agonist 4-methylhistamine (20 μM), respectively. Each agonist was used either alone or together with the respective selective antagonist as in A and B. Bars represent mean increases in NE release above basal level (± S.E.M.; $n = 12$ for C and D, respectively). Basal NE level was 279.6 ± 9.3 pmol/mg of protein ($n = 36$). ∗∗, $P < 0.01$ from corresponding control by one-way ANOVA followed by post hoc Dunnett's test.

(100 nM; 10 min) elicited a ~25 to 28% increase in NE release (Fig. 1, C and D). In the presence of the selective H₃ receptor agonist methimepip (1 nM) (Fig. 1C) or the selective H₄ receptor agonist 4-methylhistamine (20 μM) (Fig. 1D) the BNP-induced increase in NE release was reduced by ~58 and 52%, respectively (Fig. 1, C and D). These effects were abolished by the selective H₃ receptor antagonist JNJ5207852 (30 nM) (Fig. 1C) and the selective H₄ receptor antagonist A943931 (300 nM) (Fig. 1D), respectively.

These findings indicated the presence not only of histamine H₃ receptors in cardiac sympathetic nerve endings (Seyedi et al., 2005) but also H₄ receptors, both of which are capable of attenuating the release of NE elicited by K⁺-

induced depolarization or the administration of a natriuretic peptide.

BNP-Induced Dopamine Release from PC12 and PC12-H$_3$ Cells: Attenuation by Histamine H$_3$ and H$_4$ Receptor Activation. To investigate possible mechanisms of the H$_3$ and H$_4$ receptor-mediated attenuation of the NE-releasing effect of natriuretic peptides, we used the rat pheochromocytoma PC12 cell line. These cells, once differentiated with NGF, exhibit a sympathetic nerve-ending phenotype (Chan et al., 2012) and constitutively express only the H$_4$ receptor (Fig. 2). We also used a PC12 cell line stably transfected with the H$_3$ receptor (PC12-H$_3$) (Morrey et al., 2008) (Fig. 2). Dopamine is the endogenous catecholamine in both cell types (Morrey et al., 2008).

Incubation of PC12 and PC12-H$_3$ cells with BNP (100 nM, 20 min) elicited a ~48% increase in endogenous DA release (Fig. 3). In the presence of the selective H$_3$ receptor agonist methimepip (1 nM) (Fig. 3A) or the selective H$_4$ receptor agonist 4-methylhistamine (20 μM) (Fig. 3B), the BNP-induced increase in DA release was inhibited by ~90% in each case (Fig. 3). This inhibition was abolished by the selective H$_3$ receptor antagonist JNJ5207852 in PC12-H$_3$ cells (Fig. 3A) and the selective H$_4$ receptor antagonist A943931 in PC12 cells (Fig. 3B). In contrast, the H$_3$ receptor agonist methimepip, either alone or in the presence of the H$_3$ receptor antagonist JNJ5207852, failed to modify the BNP-induced increase in DA release in PC12 cells, which do not express H$_3$ receptors (i.e., negative control; Fig. 3B).
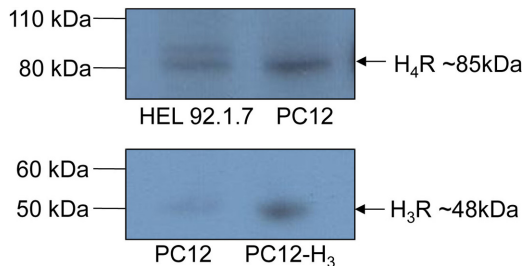

Fig. 2. Expression of histamine H$_3$ and H$_4$ receptors in PC12 and PC12-H$_3$ cells. Top, Western blotting analysis of HEL 92.1.7 cells (Liu et al., 2001; Zhu et al., 2001) (HEL 92.1.7 are human erythroleukemia cells, in which H$_4$ receptors are highly expressed, and thus serve as positive control) and PC12 cells, both expressing histamine H$_4$ receptors. Bottom, Western blotting analysis of PC12 and PC12-H$_3$ cells showing expression of histamine H$_3$ receptors in PC12-H$_3$ cells but not in PC12 cells. Twenty micrograms of total proteins were loaded in each lane.
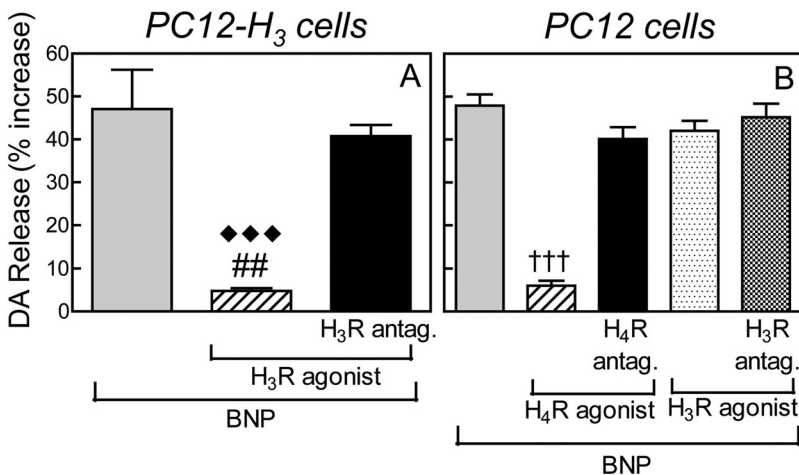
BNP Increases cAMP and Activates PKA in PC12 and PC12-H$_3$ Cells: Attenuation by Histamine H$_3$ and H$_4$ Receptor Activation. Incubation of PC12-H$_3$ cells with BNP (100 nM) caused a >2-fold increase in the intracellular concentration of cAMP (compared with a ~10-fold increase by 10 μM forskolin, positive control). The BNP-induced increase in cAMP was inhibited by ~50% by the selective H$_3$ receptor agonist methimepip (1 nM), a result that was abolished by the selective H$_3$ receptor antagonist JNJ5207852 (30 nM) (Fig. 4A). BNP also activated PKA, as evidenced by a ~60% increase in PKA phosphorylation (similar to that elicited by forskolin used as positive control; Fig. 4B). The BNP-induced increase in PKA activity was also inhibited by ~50% by the selective H$_3$ receptor agonist methimepip (1 nM), a result that was abolished by the selective H$_3$ receptor antagonist JNJ5207852 (30 nM) (Fig. 4B).

Incubation of PC12 cells with BNP (100 nM) caused a ~3-fold increase in the intracellular concentration of cAMP (compared with a ~15-fold increase by 10 μM forskolin, positive control). The BNP-induced increase in cAMP was inhibited by ~60% by the selective H$_4$ receptor agonist 4-methylhistamine (20 μM), a result that was abolished by the selective H$_4$ receptor antagonist A943931 (300 nM) (Fig. 4C). In contrast, H$_3$ receptor activation with methimepip (1 nM) did not affect the BNP-induced increase in cAMP in these cells, which did not express H$_3$ receptors (negative control) (Fig. 4C). The BNP-induced increase in PKA activity was also inhibited by ~50% by the selective H$_4$ receptor agonist 4-methylhistamine (20 μM), an effect that was abolished by the selective H$_4$ receptor antagonist A943931 (300 nM) (Fig. 4D). H$_3$ receptor activation with methimepip did not affect the BNP-induced increase in PKA activity (negative control) (Fig. 4D).

BNP Increases Intracellular Ca^{2+} in PC12 and PC12-H$_3$ Cells: Attenuation by Histamine H$_3$ and H$_4$ Receptor Activation. Depolarization of PC12 and PC12-H$_3$ cells with K$^+$ (100 mM) increased intracellular Ca^{2+} concentration ~2.5- and 5-fold, respectively (positive control). Incubation with BNP (100 nM) also increased intracellular Ca^{2+} concentration 2- and 4-fold, respectively (Fig. 5). The effect of BNP was reduced by ~40 and ~65% in the presence of the H$_4$ receptor agonist 4-methylhistamine (20 μM) and the H$_3$ receptor agonist methimepip (1 nM) in PC12 and PC12-H$_3$ cells, respectively (Fig. 5). Methimepip (negative control) did

Fig. 3. BNP-induced dopamine release in PC12 cells: inhibition by histamine H$_3$ and H$_4$ receptor activation. A, dopamine release elicited by BNP (100 nM) in PC12-H$_3$ cells, in the absence or presence of the selective H$_3$ receptor agonist methimepip (1 nM), either alone or in combination with the selective H$_3$ receptor antagonist JNJ5207852 (30 nM). Columns are means ± S.E.M. (n = 7–10). Significantly different from BNP and BNP + H$_3$ receptor agonist + H$_3$ receptor antagonist: ##, P < 0.01 and ◆◆◆, P < 0.0001, respectively, by unpaired t test. B, dopamine release elicited by BNP (100 nM) in PC12 cells, in the absence or presence of the selective H$_4$ receptor agonist 4-methyl histamine (20 μM), either alone or in combination with the selective H$_4$ receptor antagonist A943931 (300 nM). As a negative control, BNP-induced dopamine was also evaluated in PC12 cells (i.e., lacking constitutive H$_3$ receptors) in the absence or presence of the selective H$_3$ receptor agonist methimepip (1 nM), either alone or in combination with the selective H$_3$ receptor antagonist JNJ5207852 (30 nM). Columns are means (± S.E.M.; n = 8). †††, significantly different from BNP and BNP + H$_4$ receptor agonist + H$_4$ receptor antagonist (P < 0.0001 by unpaired t test).
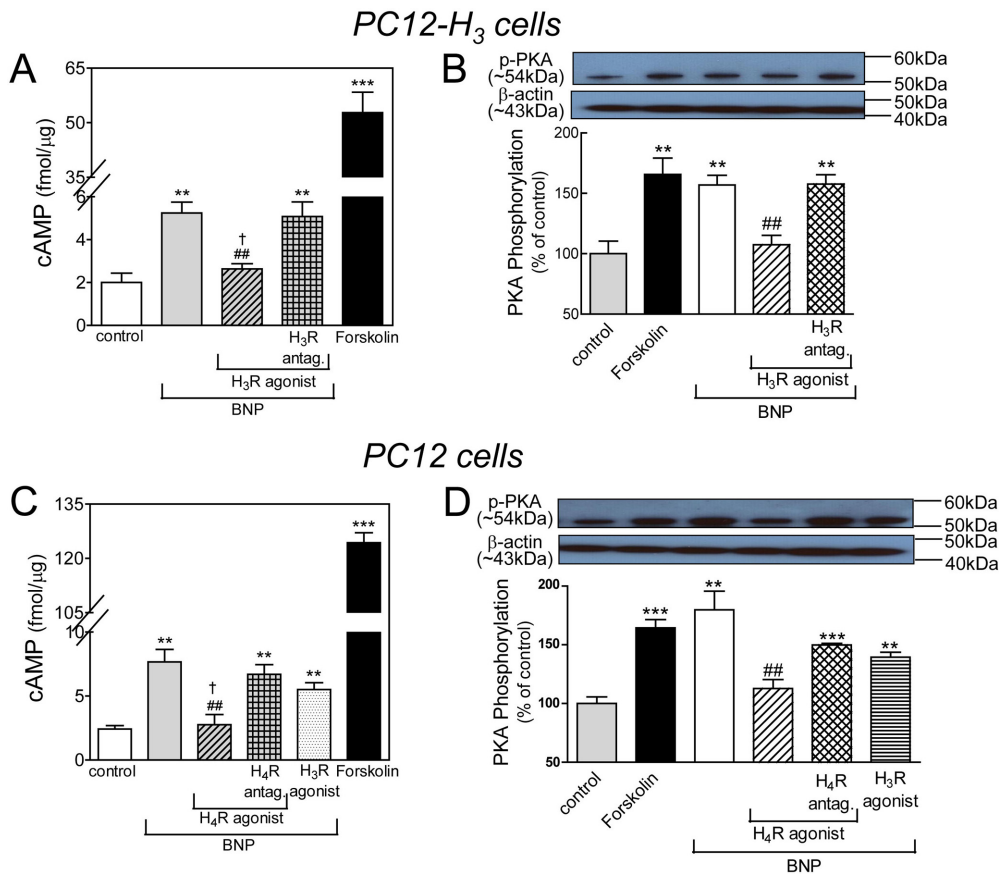
PC12-H₃ cells

PC12 cells

Fig. 4. Histamine H_3 receptor activation attenuates the increase in intracellular cAMP and phosphorylation of PKA (PKA activity) elicited by BNP in PC12-H_3 cells. Histamine H_4 receptor activation attenuates the increase in intracellular cAMP and phosphorylation of PKA elicited by BNP in PC12 cells. A, intracellular cAMP levels in PC12-H_3 cells treated with forskolin (10 μM; positive control) or BNP (100 nM) in the absence or presence of the selective H_3 receptor agonist methimepip (1 nM), either alone or in combination with the selective H_3 receptor antagonist JNJ5207852 (30 nM). Columns are means $\pm$ S.E.M. ($n = 4$). Significantly different from control: ∗∗, $P < 0.01$ and ∗∗∗, $P < 0.001$ by unpaired t test. Significantly different from BNP: ##, $P < 0.01$ by unpaired t test. Significantly different from BNP + H_3 receptor agonist + H_3 receptor antagonist: †, $P < 0.05$ by unpaired t test. B, PKA activity in PC12-H_3 cells treated with forskolin (10 μM; positive control) or BNP (100 nM) in the absence or presence of the H_3 receptor agonist methimepip (1 nM), either alone or in combination with the H_3 receptor antagonist JNJ5207852 (30 nM). Top band, representative immunoblot of PC12-H_3 cell lysate probed with antiphosphorylated PKA antibody. Bottom band, same immunoblot probed with anti-β-actin antibody. Bars represent mean quantitative values ($\pm$ S.E.M.; $n = 4$). Significantly different from control: ∗∗, $P < 0.01$ by unpaired t test. Significantly different from BNP and BNP + H_3 receptor agonist + H_3 receptor antagonist: ##, $P < 0.01$ by unpaired t test. C, intracellular cAMP levels in PC12 cells treated with forskolin (10 μM; positive control) or BNP (100 nM) in the absence or presence of the H_3 receptor agonist methimepip (1 nM; negative control) or H_4 receptor agonist 4-methylhistamine (20 μM), either alone or in combination with the H_4 receptor antagonist A943931 (300 nM). Columns are means $\pm$ S.E.M. ($n = 4$). Significantly different from control: ∗∗, $P < 0.01$ and ∗∗∗, $P < 0.0001$ by unpaired t test. Significantly different from BNP: ##, $P < 0.01$ by unpaired t test. Significantly different from BNP + H_4 receptor agonist + H_4 receptor antagonist: †, $P < 0.05$ by unpaired t test. D, PKA activity in PC12 cells treated with forskolin (10 μM; positive control) or BNP (100 nM) in the absence or presence of the H_3 receptor agonist methimepip (1 nM) or H_4 receptor agonist 4-methylhistamine (20 μM), either alone or in combination with the H_4 receptor antagonist A943931 (300 nM). Top band, representative immunoblot of PC12 cell lysate probed with antiphosphorylated PKA antibody. Bottom band, same immunoblot probed with anti-β-actin antibody. Bars represent mean quantitative values ($\pm$ S.E.M.; $n = 4$). Significantly different from control: ∗∗∗, $P < 0.001$ and ∗∗, $P < 0.01$ by unpaired t test. Significantly different from BNP and BNP + H_4 receptor agonist + H_4 receptor antagonist: ##, $P < 0.01$ by unpaired t test.

not affect the BNP-induced increase in intracellular Ca^{2+} in PC12 cells (Fig. 5B). The H_3 and H_4 receptor-mediated inhibition of the BNP-induced increase in intracellular Ca^{2+} was abolished by the respective H_3 and H_4 receptor antagonists [JNJ5207852 (30 nM) and A943931 (300 nM)] (Fig. 5).

BNP-Induced Increase in PKG Activity in PC12 and PC12-H_3 Cells: Attenuation by Histamine H_3 and H_4 Receptor Activation. Incubation of PC12-H_3 cells with either 8-Br-cGMP (1 μM; positive control) or BNP (100 nM) elicited a 2-fold increase in PKG activity, which was prevented either by the PKG inhibitor Rp-8-Br-cGMPS (0.5 μM) (Moretto et al., 1993) or the H_3 receptor agonist methimepip (1 nM); methimepip's effect was abolished by the H_3 receptor antagonist JNJ5207852 (30 nM) (Fig. 6A). Incubation of PC12 cells with either 8-Br-cGMP (1 μM; positive control) or

BNP (100 nM) elicited a ~50% increase in PKG activity, which was prevented either by the PKG inhibitor Rp-8-Br-cGMPS (0.5 μM) or the H_4 receptor agonist 4-methylhistamine (20 μM); 4-methylhistamine's effect was abolished by the H_4 receptor antagonist A943931 (300 nM) (Fig. 6B). Methimepip did not affect the BNP-induced increase in PKG activity in PC12 cells, which do not constitutively express H_3 receptors (negative control) (Fig. 6B).

To further assess the role of diminished PKG activity in the H_3 and H_4 receptor-mediated attenuation of BNP-induced catecholamine exocytosis, we next determined whether a synergistic effect could be seen when H_3 and H_4 receptor activation was combined with PKG inhibition. As shown in Fig. 6C, when either methimepip or Rp-8-Br-cGMPS was used at subthreshold concentrations (0.03 nM and 0.3 μM, respectively), neither
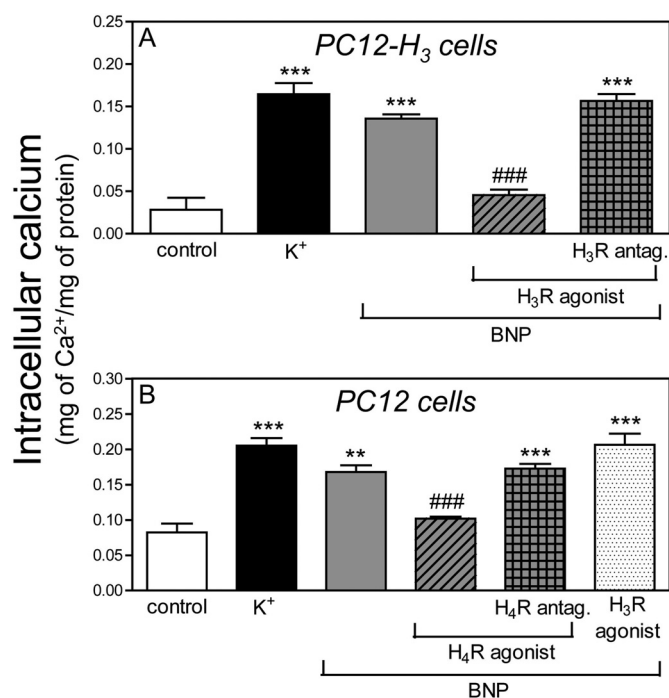
Fig. 5. Histamine H$_3$ and H$_4$ receptor activation attenuates the increase in intracellular Ca^{2+} elicited by BNP in PC12-H$_3$ and PC12 cells, respectively. A, intracellular Ca^{2+} content of PC12-H$_3$ cells depolarized with K$^+$ (100 mM; positive control) or BNP (100 nM) in the absence or presence of the H$_3$ receptor agonist methimepip (1 nM), either alone or in combination with the H$_3$ receptor antagonist JNJ5207852 (30 nM). Bars are means ± S.E.M. ($n = 4$). Significantly different from control: ***, $P < 0.001$ by unpaired t test. Significantly different from BNP and BNP + H$_3$ receptor agonist + H$_3$ receptor antagonist: ###, $P < 0.001$ by unpaired t test. B, intracellular Ca^{2+} content of PC12 cells depolarized with K$^+$ (100 mM; positive control) or BNP (100 nM) in the absence or presence of the H$_4$ receptor agonist 4-methyl histamine (20 μM), either alone or in combination with the H$_4$ receptor antagonist A943931 (300 nM). The H$_3$ receptor agonist methimepip (1 nM) was used as a negative control. Bars are means ± S.E.M. ($n = 4$). Significantly different from control: **, $P < 0.01$ and ***, $P < 0.001$ by unpaired t test. Significantly different from BNP and BNP + H$_4$R agonist + H$_4$R antagonist: ###, $P < 0.001$ by unpaired t test.

caused a significant diminution of BNP-induced (100 nM) NE release in cardiac synaptosomes. In contrast, a significant attenuation occurred when the same subthreshold concentrations of methimepip and Rp-8-Br-cGMPS were combined (Fig. 6C). Likewise, when either 4-methylhistamine or Rp-8-Br-cGMPS was used at subthreshold concentrations (0.03 and 0.3 μM, respectively), neither caused a significant diminution of BNP-induced (100 nM) NE release in cardiac synaptosomes. In contrast, a significant attenuation occurred when the same subthreshold concentrations of 4-methylhistamine and Rp-8-Br-cGMPS were combined (Fig. 6D). These synergistic responses suggested that a decrease in PKG activity is likely to be involved in the H$_3$ and H$_4$ receptor-mediated attenuation of BNP-induced catecholamine exocytosis.

Histamine H$_3$ and H$_4$ Receptor Activation Prevents the BNP-Induced Inhibition of PDE3 Activity in PC12 Cells. Incubation of PC12 and PC12-H$_3$ cells with BNP (100 nM) significantly decreased the rate of cAMP hydrolysis (an index of PDE3 activity). Insulin (100 nM) (Watanabe et al., 2004) and cilostamide (10 μM) (Hidaka et al., 1979), PDE3 activator and inhibitor, respectively, served as controls (Fig. 7, A and B). In the presence of the selective H$_3$ receptor agonist methimepip (1 nM), the BNP-induced decrease in PDE3 activ-

ity was reversed in PC12-H$_3$ cells, and this action was abolished by the selective H$_3$ receptor antagonist JNJ5207852 (30 nM) (Fig. 7A). Likewise, in the presence of the selective H$_4$ receptor agonist 4-methylhistamine (20 μM), the BNP-induced decrease in PDE3 activity cells was reversed in PC12 cells, and this action was abolished by the selective H$_4$ receptor antagonist A943931 (300 nM) (Fig. 7B). In contrast, methimepip did not modify the BNP-induced decrease in PDE3 activity in PC12 cells, because these cells do not constitutively express H$_3$ receptors (negative control) (Fig. 7B).

To further assess the role of an increased PDE3 activity in the H$_3$ and H$_4$ receptor-mediated attenuation of BNP-induced catecholamine exocytosis, we next determined whether a synergistic effect could be seen when H$_3$ and H$_4$ receptor activation was combined with PDE3 stimulation. As shown in Fig. 7C, when either methimepip or insulin was used at subthreshold concentrations (0.03 and 10 nM, respectively), neither caused a significant diminution of BNP-induced (100 nM) NE release in cardiac synaptosomes. In contrast, a significant attenuation occurred when the same subthreshold concentrations of methimepip and insulin were combined (Fig. 7C). Likewise, when either 4-methylhistamine or insulin was used at subthreshold concentrations (0.03 μM and 10 nM, respectively), neither caused a significant diminution of BNP-induced (100 nM) NE release in cardiac synaptosomes. In contrast, a significant attenuation occurred when the same subthreshold concentrations of 4-methylhistamine and insulin were combined (Fig. 7D). These synergistic responses suggested that an increase in PDE3 activity is likely to be involved in the H$_3$ and H$_4$ receptor-mediated attenuation of BNP-induced catecholamine exocytosis.

Discussion

The purpose of our study was to search for novel means to prevent the recently uncovered proadrenergic effects of natriuretic peptides. Our findings indicate that the activation of neuronal histamine H$_3$ and H$_4$ receptors attenuates BNP-induced catecholamine release by inhibiting PKG, thus enhancing PDE3-mediated cAMP metabolism culminating in a decrease in intracellular Ca^{2+}.

Although H$_4$ receptors are expressed predominantly in hematopoietic cells (Nijmeijer et al., 2012), their presence had been reported in the brain (Zhu et al., 2001; Connelly et al., 2009) and peripheral neurons of the nasal mucosa (Nakaya et al., 2004). Here, we functionally identified H$_4$ receptors in cardiac sympathetic neurons and demonstrated their protein expression in NGF-differentiated PC12 cells exhibiting a sympathetic neuron phenotype. It is noteworthy that differentiated PC12 cells constitutively expressed only H$_4$ receptors. We further demonstrated that, similar to H$_3$ receptors, these neuronal H$_4$ receptors negatively modulate catecholamine exocytosis elicited by K$^+$-induced depolarization or BNP. Given that H$_4$ receptors are highly homologous to H$_3$ receptors and that, like H$_3$ receptors, are coupled to inhibitory G$_{i/o}$ proteins (Oda et al., 2000; Liu et al., 2001; Zhu et al., 2001), it was not surprising to find that H$_4$ receptors attenuate catecholamine exocytosis elicited by K$^+$ depolarization. As is the case for H$_3$ receptors, the antiexocytotic action of H$_4$ receptors could result from a Gα_i-mediated impairment of the adenylyl cyclase-cAMP-PKA pathway lead-
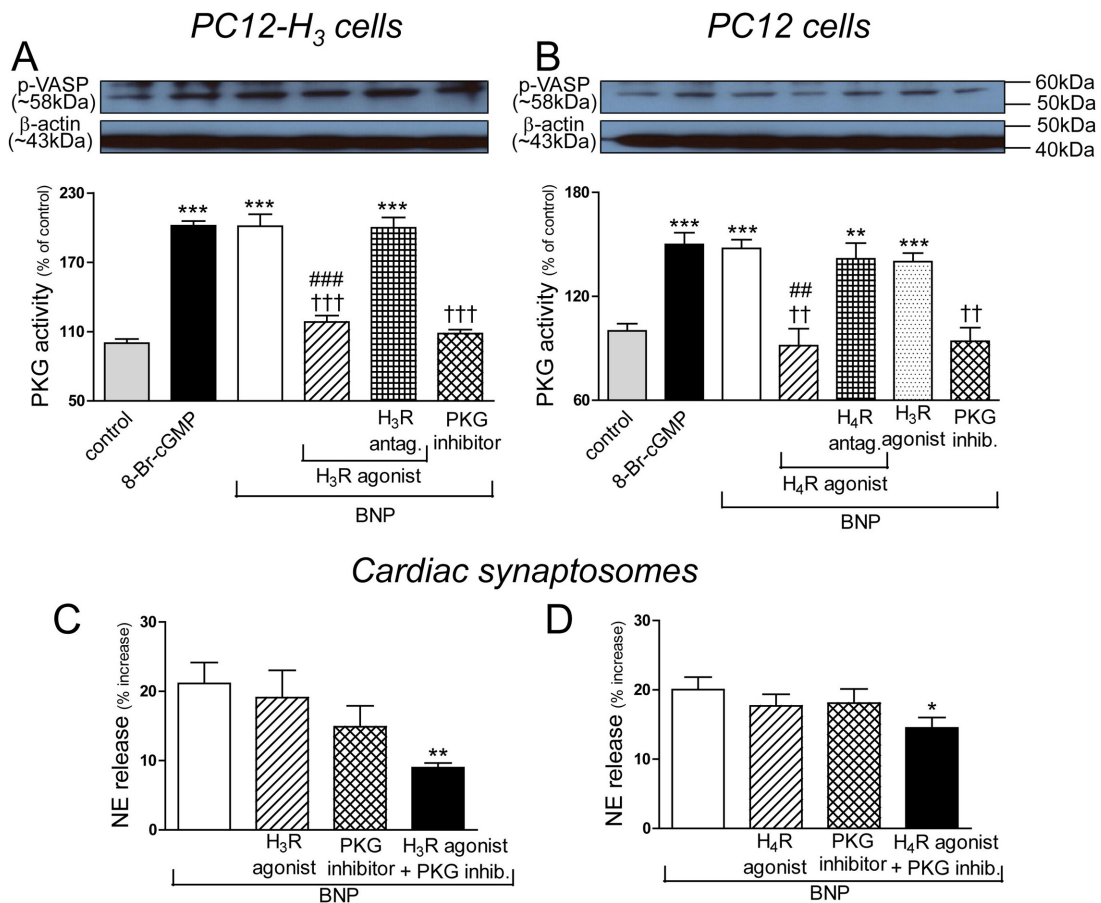
Fig. 6. A and B, histamine H_3- and H_4 receptor activation inhibits the increase in PKG activity elicited by BNP in PC12-H_3 and PC12 cells, respectively. C and D, H_3 and H_4 receptor activation synergizes with PKG inhibition in attenuating BNP-induced NE release in cardiac synaptosomes, respectively. A, PKG activity in PC12-H_3 cells treated with 8-Br-cGMP (1 μM; positive control) or BNP (100 nM) in the absence or presence of the PKG inhibitor Rp-8-Br-cGMPS (0.5 μM) or the H_3 receptor agonist methimepip (1 nM), either alone or in combination with the H_3 receptor antagonist JNJ5207852 (30 nM). Top band, representative immunoblot of PC12-H_3 cell lysate probed with antiphosphorylated VASP (p-VASP; a major PKG substrate) antibody. Bottom band, same immunoblot probed with anti-β-actin antibody. Bars represent mean quantitative values ($\pm$ S.E.M.; $n = 4$). Significantly different from control: ***, $P < 0.0001$ by unpaired t test. Significantly different from BNP: †††, $P < 0.001$ by unpaired t test. Significantly different from BNP + H_3 receptor agonist + H_3 receptor antagonist: ###, $P < 0.001$ by unpaired t test. B, PKG activity in PC12 cells treated with 8-Br-cGMP (1 μM; positive control) or BNP (100 nM) in the absence or presence of the PKG inhibitor Rp-8-Br-cGMPS (0.5 μM) or the H_4 receptor agonist (4-methylhistamine; 20 μM), either alone or in combination with the H_4 receptor antagonist (A943931; 300 nM). The H_3 receptor agonist methimepip (1 nM; negative control) failed to modify the response to BNP in PC12 cells, which do not constitutively express H_3 receptors. Top band, representative immunoblot of PC12 cell lysate probed with antiphosphorylated VASP antibody. Bottom band, same immunoblot probed with anti-β-actin antibody. Bars represent mean quantitative values ($\pm$ S.E.M.; $n = 4$). Significantly different from control: ***, $P < 0.001$ and **, $P < 0.01$ by unpaired t test. Significantly different from BNP: ††, $P < 0.01$ by unpaired t test. Significantly different from BNP + H_4R agonist + H_4R antagonist: ##, $P < 0.01$ by unpaired t test. C, inhibition of BNP (100 nM)-induced NE release in cardiac synaptosomes by subthreshold concentrations of the PKG inhibitor Rp-8-Br-cGMPS (0.3 μM) and the H_3 receptor agonist methimepip (0.03 nM), administered either alone or in combination. D, inhibition of BNP-induced NE release in cardiac synaptosomes by subthreshold concentrations of the PKG inhibitor Rp-8-Br-cGMPS and the H_4 receptor agonist 4-methyl histamine (0.03 μM), administered either alone or in combination. Note in C and D that a significant attenuation of NE release occurs when the PKG inhibitor is combined either with the H_3 or the H_4 receptor agonist (*, $P < 0.05$ and **, $P < 0.005$ by unpaired t test). Bars, means $\pm$ S.E.M. ($n = 8$–18), represent the BNP-induced increase in NE release above the basal level of 232.1 $\pm$ 8.9 pmol/mg ($n = 25$).

ing to a decrease in intraneuronal Ca^{2+} (Silver et al., 2002; Seyedi et al., 2005). A direct $G\beta\gamma$-induced attenuation of Ca^{2+} current (I_{Ca}) could also play a role (Morrey et al., 2008). On the other hand, regarding the H_3 and H_4 receptor-mediated attenuation of catecholamine release elicited by BNP, our findings suggest that the reduction in intracellular Ca^{2+} derives primarily from PKG inhibition and a consequent enhancement in PDE3-mediated cAMP metabolism, rather than a $G\alpha_i$-mediated decrease in adenylyl cyclase activity.

We found that H_3 and H_4 receptor activation synergized with PKG inhibition and PDE3 stimulation, respectively, in inhibiting the BNP-induced promotion of catecholamine release. These synergistic responses strongly suggest that a decrease in PKG activity and an increase in PDE3 activity

are both pivotal in the H_3 and H_4 receptor-mediated attenuation of BNP-induced catecholamine exocytosis. Whether PDE3 activation indirectly results from PKG inhibition because of H_3 and H_4 receptor activation or is a direct and independent target of H_3 and H_4 receptor activation remains to be understood.

We can only speculate at this point on the molecular mechanisms possibly involved in H_3 and H_4 receptor-mediated PKG inhibition and PDE3 stimulation. We had shown previously that imetit, a mixed H_3/H_4 receptor agonist (Morse et al., 2001; Zhu et al., 2001), attenuates the phorbol ester-induced activation of PKC in NGF-differentiated PC12-H_3 cells, an action prevented by the mixed H_3/H_4 receptor antagonist clobenpropit (Hashikawa-Hobara et al., 2012). Be-
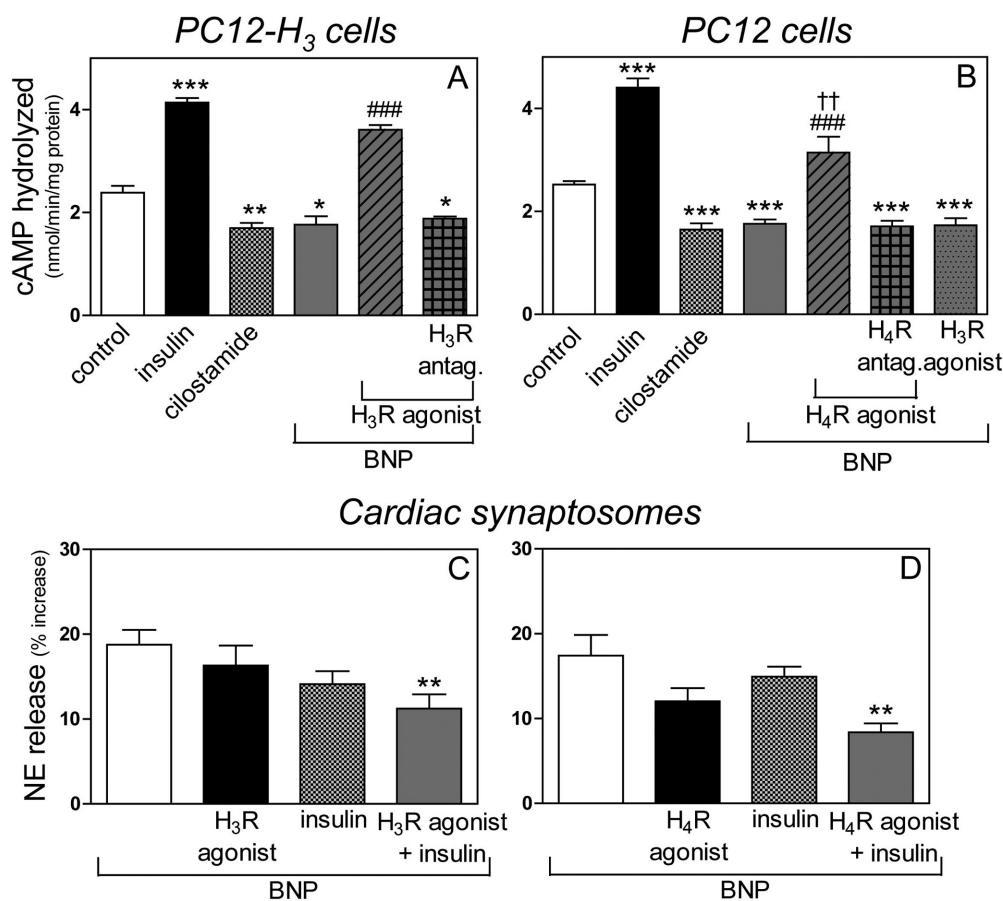
Fig. 7. A and B, histamine H$_3$ and H$_4$ receptor activation inhibits the decrease in PDE3 activity (expressed as rate of cAMP hydrolyzed) elicited by BNP in PC12-H$_3$ and PC12 cells. C and D, H$_3$ and H$_4$ receptor activation synergizes with PDE3 activation in attenuating BNP-induced NE release in cardiac synaptosomes. A, BNP (100 nM) decreases the rate of cAMP hydrolyzed (i.e., a decrease in PDE3 activity) in PC12-H$_3$ cells. The H$_3$ receptor agonist methimepip (1 nM) reverses the PDE3-inhibiting effect of BNP. Pretreatment with the H$_3$ receptor antagonist JNJ5207852 (30 nM) restores the PDE3-inhibiting effect of BNP. The PDE3 activator insulin (100 nM) and the PDE3 inhibitor cilostamide (10 μM) serve as controls. Columns are means ± S.E.M. ($n = 4$). Significantly different from control: ∗∗∗, $P < 0.001$; ∗∗, $P < 0.01$; and∗, $P < 0.05$ by unpaired t test. Significantly different from BNP and BNP + H$_3$ receptor agonist + H$_3$ receptor antagonist: ###, $P < 0.0001$ by unpaired t test. B, BNP (100 nM) decreases PDE3 activity in PC12 cells. The H$_4$ receptor agonist 4-methylhistamine (20 μM) reverses the PDE3-inhibiting effect of BNP. Pretreatment with the H$_4$ receptor antagonist A943931 (300 nM) restores the PDE3-inhibiting effect of BNP. Note that the H$_3$R agonist methimepip (1 nM) does not affect the PDE3-inhibiting effect of BNP, because PC12 cells do not constitutively express H$_3$ receptors (negative control). As in A, the PDE3 activator insulin (100 nM) and the PDE3 inhibitor cilostamide (10 μM) serve as controls. Bars are means ± S.E.M. ($n = 4$–14). Significantly different from control: ∗∗∗, $P < 0.0001$ by unpaired t test. Significantly different from BNP: ###, $P < 0.0001$ by unpaired t test. Significantly different from BNP + H$_4$ receptor agonist + H$_4$ receptor antagonist: ††, $P < 0.01$ by unpaired t test. C, inhibition of NE release induced by BNP (100 nM) in cardiac synaptosomes by subthreshold concentrations of the PDE3 activator insulin (10 nM) and the H$_3$ receptor agonist methimepip (0.03 nM). D, inhibition of NE release induced by BNP (100 nM) in cardiac synaptosomes by subthreshold concentrations of the PDE3 activator insulin (10 nM) and the H$_4$ receptor agonist 4-methylhistamine (0.03 μM) administered either alone or in combination. Note in C and D that a significant attenuation of NE release occurs when insulin is combined either with the H$_3$ or the H$_4$ receptor agonist (∗∗, $P < 0.005$ by unpaired t test). Bars, means ± S.E.M. ($n = 8$–19), represent BNP-induced increase in NE release above the basal level of 226.9 ± 12.9 pmol/mg ($n = 21$).

cause PKC stimulation has been reported previously to activate PKG (Hou et al., 2003), it is conceivable that the H$_3$/H$_4$ receptor-induced decrease in PKC activity could in turn reduce PKG activity in cardiac sympathetic neurons. Reduced PKG activity would then alleviate PKG-mediated PDE3 inhibition, augment cAMP hydrolysis, and ultimately decrease intracellular Ca^{2+} and NE exocytosis. It is also possible that the activation of H$_3$ and H$_4$ receptors may lead to PDE3 stimulation independently of PKG inhibition. In fact, H$_3$ receptors are known to activate the phosphatidylinositol 3-kinase pathway, which results in the phosphorylation/activation of Akt (Leurs et al., 2005). Akt is involved in the phosphorylation/activation of PDE3B (Wijkander et al., 1998), which was shown to be expressed in heart tissue with PDE3A (Liu and Maurice, 1998). Given the high homology of H$_3$ receptors to H$_4$ receptors, and the fact that they both are

G$_{i/o}$-coupled (Nijmeijer et al., 2012), it is conceivable that H$_3$ and H$_4$ receptor activation may lead via the phosphatidylinositol 3-kinase pathway to PDE3 stimulation, increased cAMP hydrolysis, and decreased NE release.

It is noteworthy that PDE3 activity is significantly reduced in failing human hearts and murine hearts with chronic pressure overload (Ding et al., 2005). Moreover, long-term inhibition of PDE3 has been found to be associated with a 40% increase in mortality, primarily as a result of arrhythmias and sudden death (Packer et al., 1991; Nony et al., 1994). We had reported that inhibition of PDE3-mediated cAMP hydrolysis by natriuretic peptides, at concentrations likely to be reached in advanced congestive heart failure, promote excessive NE release (Chan et al., 2012), which we contend could explain at least in part why natriuretic peptides failed to correct the symptoms of congestive heart failure (O'Connor et al., 2011). Thus, we had

advocated that agents that preserve PDE3 function, rather than inhibiting it, may be beneficial in the treatment of cardiac dysfunctions associated with excessive sympathetic activity (Chan et al., 2012). We report here that histamine H_3 and H_4 receptor activation stimulates PDE3 activity via PKG inhibition and/or directly. Accordingly, preserving and/or stimulating PDE3 function via H_3 and H_4 receptor activation could offer a useful new approach to the treatment of cardiac dysfunctions with natriuretic peptides. Indeed, although β-adrenoceptor blockade has been advocated to prevent the deleterious effects of chronic BNP exposure in congestive heart failure (Thireau et al., 2012), stimulation of PDE3 activity via H_3 and H_4 receptor activation might be preferable, given the notorious adverse effects of β-blockers (Lewis and McDevitt, 1986).

In conclusion, we had reported previously that natriuretic peptides augment NE exocytosis from cardiac sympathetic neurons by a PKG-mediated inhibition of PDE3 activity, which results sequentially in an increase in intraneuronal cAMP, augmented PKA activity, phosphorylation of Ca^{2+} channels, and increased intracellular Ca^{2+} (Chan et al., 2012). We present new evidence that this pathway can be effectively interrupted at the PKG and PDE3 levels. Indeed, our findings indicate that PKG and PDE3 are targeted for inhibition and stimulation, respectively, when histamine H_3 and H_4 receptors are activated (see Fig. 8).

Cardiac sympathetic overstimulation is characteristic of advanced heart failure (Esler and Kaye, 2000; Braunwald, 2008; Grassi et al., 2009), which was recently found not to be improved by the administration of recombinant BNP (nesiritide) (O'Connor et al., 2011), despite the predicated beneficial effects of natriuretic peptides (Molkentin, 2003; Munagala et al., 2004). Because excessive NE release is likely to offset the

desirable effects of natriuretic peptides, our findings suggest novel means to alleviate their adverse effects and improve their therapeutic potential.

Acknowledgments

Authorship Contributions

Participated in research design: Chan, Robador, and Levi.
Conducted experiments: Chan and Robador.
Performed data analysis: Chan, Robador, and Levi.
Wrote or contributed to the writing of the manuscript: Chan, Robador, and Levi.

References

Barbier AJ, Berridge C, Dugovic C, Laposky AD, Wilson SJ, Boggs J, Aluisio L, Lord B, Mazur C, Pudiak CM, et al. (2004) Acute wake-promoting actions of JNJ-5207852, a novel, diamine-based H_3 antagonist. *Br J Pharmacol* **143:**649–661.
Braunwald E (2008) Biomarkers in heart failure. *N Engl J Med* **358:**2148–2159.
Chan NY, Seyedi N, Takano K, and Levi R (2012) An unsuspected property of natriuretic peptides: promotion of calcium-dependent catecholamine release via protein kinase G-mediated phosphodiesterase type 3 inhibition. *Circulation* **125:**298–307.
Connelly WM, Shenton FC, Lethbridge N, Leurs R, Waldvogel HJ, Faull RL, Lees G, and Chazot PL (2009) The histamine H4 receptor is functionally expressed on neurons in the mammalian CNS. *Br J Pharmacol* **157:**55–63.
Cowart MD, Altenbach RJ, Liu H, Hsieh GC, Drizin I, Milicic I, Miller TR, Witte DG, Wishart N, Fix-Stenzel SR, et al. (2008) Rotationally constrained 2,4-diamino-5,6-disubstituted pyrimidines: a new class of histamine H4 receptor antagonists with improved druglikeness and in vivo efficacy in pain and inflammation models. *J Med Chem* **51:**6547–6557.
Ding B, Abe J, Wei H, Huang Q, Walsh RA, Molina CA, Zhao A, Sadoshima J, Blaxall BC, Berk BC, et al. (2005) Functional role of phosphodiesterase 3 in cardiomyocyte apoptosis: implication in heart failure. *Circulation* **111:**2469–2476.
Esler M and Kaye D (2000) Measurement of sympathetic nervous system activity in heart failure: the role of norepinephrine kinetics. *Heart Fail Rev* **5:**17–25.
Fujimura M, Akaike M, Iwase T, Yoshida S, Sumitomo Y, Yagi S, Ikeda Y, Hashizume S, Aihara K, Nishiuchi T, et al. (2009) Decrease in plasma brain natriuretic peptide level in the early phase after the start of carvedilol therapy is a novel predictor of long-term outcome in patients with chronic heart failure. *Acta Cardiol* **64:**589–595.
Gill RM, Braz JC, Jin N, Etgen GJ, and Shen W (2007) Restoration of impaired endothelium-dependent coronary vasodilation in failing heart: role of eNOS phosphorylation and CGMP/cGK-I signaling. *Am J Physiol Heart Circ Physiol* **292:**H2782–H2790.
Grassi G, Seravalle G, Quarti-Trevano F, and Dell'oro R (2009) Sympathetic activation in congestive heart failure: evidence, consequences and therapeutic implications. *Curr Vasc Pharmacol* **7:**137–145.
Hashikawa-Hobara N, Chan NY, and Levi R (2012) Histamine 3 receptor activation reduces the expression of neuronal angiotensin II type 1 receptors in the heart. *J Pharmacol Exp Ther* **340:**185–191.
Hidaka H, Hayashi H, Kohri H, Kimura Y, Hosokawa T, Igawa T, and Saitoh Y (1979) Selective inhibitor of platelet cyclic adenosine monophosphate phosphodiesterase, cilostamide, inhibits platelet aggregation. *J Pharmacol Exp Ther* **211:**26–30.
Hou Y, Lascola J, Dulin NO, Ye RD, and Browning DD (2003) Activation of cGMP-dependent protein kinase by protein kinase C. *J Biol Chem* **278:**16706–16712.
Imamura M, Seyedi N, Lander HM, and Levi R (1995) Functional identification of histamine H_3 receptors in the human heart. *Circ Res* **77:**206–210.
Kitbunnadaj R, Hashimoto T, Poli E, Zuiderveld OP, Menozzi A, Hidaka R, de Esch IJ, Bakker RA, Menge WM, Yamatodani A, et al. (2005) N-substituted piperidinyl alkyl imidazoles: discovery of methimepip as a potent and selective histamine H_3 receptor agonist. *J Med Chem* **48:**2100–2107.
Leurs R, Bakker RA, Timmerman H, and de Esch IJ (2005) The histamine H_3 receptor: from gene cloning to H_3 receptor drugs. *Nat Rev Drug Discov* **4:**107–120.
Levi R and Smith NC (2000) Histamine H_3 receptors: a new frontier in myocardial ischemia. *J Pharmacol Exp Ther* **292:**825–830.
Lewis RV and McDevitt DG (1986) Adverse reactions and interactions with β-adrenoceptor blocking drugs. *Med Toxicol* **1:**343–361.
Lim HD, van Rijn RM, Ling P, Bakker RA, Thurmond RL, and Leurs R (2005) Evaluation of histamine H1, H2, and H3 receptor ligands at the human histamine H4 receptor: identification of 4-methylhistamine as the first potent and selective histamine H4 receptor agonist. *J Pharmacol Exp Ther* **314:**1310–1321.
Liu C, Ma X, Jiang X, Wilson SJ, Hofstra CL, Blevitt J, Pyati J, Li X, Chai W, Carruthers N, et al. (2001) Cloning and pharmacological characterization of a fourth histamine receptor (H₄) expressed in bone marrow. *Mol Pharmacol* **59:**420–426.
Liu H and Maurice DH (1998) Expression of cyclic GMP-inhibited phosphodiesterases 3A and 3B (PDE3A and PDE3B) in rat tissues: differential subcellular localization and regulated expression by cyclic AMP. *Br J Pharmacol* **125:**1501–1510.
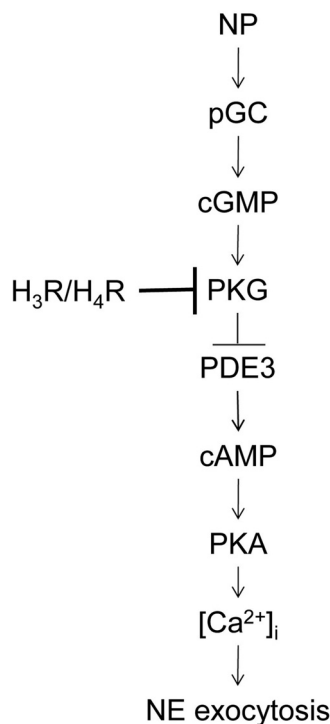Meredith IT, Broughton A, Jennings GL, and Esler MD (1991) Evidence of a selective

Fig. 8. Histamine H_3 and H_4 receptor activation inhibits Ca^{2+}-dependent NE exocytosis from cardiac sympathetic nerves via inhibition of PKG and consequent reduction of PKG-dependent PDE3 inhibition. NP, natriuretic peptides; pGC, particulate guanylyl cyclase; cGMP, cyclic GMP; $[Ca^{2+}]_i$, intracellular calcium.

increase in cardiac sympathetic activity in patients with sustained ventricular arrhythmias. *N Engl J Med* **325**:618–624.

Molkentin JD (2003) A friend within the heart: natriuretic peptide receptor signaling. *J Clin Invest* **111**:1275–1277.

Moretto M, López FJ, and Negro-Vilar A (1993) Nitric oxide regulates luteinizing hormone-releasing hormone secretion. *Endocrinology* **133**:2399–2402.

Morrey C, Estephan R, Abbott GW, and Levi R (2008) Cardioprotective effect of histamine H₃ receptor activation: pivotal role of Gβγ-dependent inhibition of voltage-operated Ca²⁺ channels. *J Pharmacol Exp Ther* **326**:871–878.

Morse KL, Behan J, Laz TM, West RE Jr, Greenfeder SA, Anthes JC, Umland S, Wan Y, Hipkin RW, Gonsiorek W, et al. (2001) Cloning and characterization of a novel human histamine receptor. *J Pharmacol Exp Ther* **296**:1058–1066.

Munagala VK, Burnett JC Jr, and Redfield MM (2004) The natriuretic peptides in cardiovascular medicine. *Curr Probl Cardiol* **29**:707–769.

Nakaya M, Takeuchi N, and Kondo K (2004) Immunohistochemical localization of histamine receptor subtypes in human inferior turbinates. *Ann Otol Rhinol Laryngol* **113**:552–557.

Nijmeijer S, de Graaf C, Leurs R, and Vischer HF (2012) Molecular pharmacology of histamine H4 receptors. *Front Biosci* **17**:2089–2106.

Nony P, Boissel JP, Lievre M, Leizorovicz A, Haugh MC, Fareh S, and de Breyne B (1994) Evaluation of the effect of phosphodiesterase inhibitors on mortality in chronic heart failure patients. A meta-analysis. *Eur J Clin Pharmacol* **46**:191–196.

O'Connor CM, Starling RC, Hernandez AF, Armstrong PW, Dickstein K, Hasselblad V, Heizer GM, Komajda M, Massie BM, McMurray JJ, et al. (2011) Effect of nesiritide in patients with acute decompensated heart failure. *N Engl J Med* **365**:32–43.

Oda T, Morikawa N, Saito Y, Masuho Y, and Matsumoto S (2000) Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J Biol Chem* **275**:36781–36786.

Packer M, Carver JR, Rodeheffer RJ, Ivanhoe RJ, DiBianco R, Zeldis SM, Hendrix GH, Bommer WJ, Elkayam U, and Kukin ML. (1991) Effect of oral milrinone on mortality in severe chronic heart failure. The PROMISE Study Research Group. *N Engl J Med* **325**:1468–1475.

Schömig A (1990) Catecholamines in myocardial ischemia. Systemic and cardiac release. *Circulation* **82**:II13–II22.

Seyedi N, Mackins CJ, Machida T, Reid AC, Silver RB, and Levi R (2005) Histamine H₃ receptor-induced attenuation of norepinephrine exocytosis: a decreased PKA activity mediates a reduction in intracellular calcium. *J Pharmacol Exp Ther* **312**:272–280.

Seyedi N, Win T, Lander HM, and Levi R (1997) Bradykinin B₂ receptor activation augments norepinephrine exocytosis from cardiac sympathetic nerve endings. Mediation by autocrine/paracrine mechanisms. *Circ Res* **81**:774–784.

Silver RB, Poonwasi KS, Seyedi N, Wilson SJ, Lovenberg TW, and Levi R (2002) Decreased intracellular calcium mediates the histamine H₃ receptor-induced attenuation of norepinephrine exocytosis from cardiac sympathetic nerve endings. *Proc Natl Acad Sci U S A* **99**:501–506.

Simon T, Becker R, Voss F, Bikou O, Hauck M, Licka M, Katus HA, and Bauer A (2008) Elevated B-type natriuretic peptide levels in patients with nonischemic cardiomyopathy predict occurrence of arrhythmic events. *Clin Res Cardiol* **97**:306–309.

Springer J, Azer J, Hua R, Robbins C, Adamczyk A, McBoyle S, Bissell MB, and Rose RA (2012) The natriuretic peptides BNP and CNP increase heart rate and electrical conduction by stimulating ionic currents in the sinoatrial node and atrial myocardium following activation of guanylyl cyclase-linked natriuretic peptide receptors. *J Mol Cell Cardiol* **52**:1122–1134.

Thireau J, Karam S, Fauconnier J, Roberge S, Cassan C, Cazorla O, Aimond F, Lacampagne A, Babuty D, and Richard S (2012) Functional evidence for an active role of B-type natriuretic peptide in cardiac remodelling and pro-arrhythmogenicity. *Cardiovasc Res* **95**:59–68.

Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Omland T, Wolf PA, and Vasan RS (2004) Plasma natriuretic peptide levels and the risk of cardiovascular events and death. *N Engl J Med* **350**:655–663.

Watanabe T, Satoo H, Kohara K, Takami R, Motoyashiki T, Morita T, and Ueki H (2004) Orthovanadate stimulates cAMP phosphodiesterase 3 activity in isolated rat hepatocytes through mitogen-activated protein kinase activation dependent on cAMP-dependent protein kinase. *Biol Pharm Bull* **27**:789–796.

Wijkander J, Landström TR, Manganiello V, Belfrage P, and Degerman E (1998) Insulin-induced phosphorylation and activation of phosphodiesterase 3B in rat adipocytes: possible role for protein kinase B but not mitogen-activated protein kinase or p70 S6 kinase. *Endocrinology* **139**:219–227.

Zhu Y, Michalovich D, Wu H, Tan KB, Dytko GM, Mannan IJ, Boyce R, Alston J, Tierney LA, Li X, et al. (2001) Cloning, expression, and pharmacological characterization of a novel human histamine receptor. *Mol Pharmacol* **59**:434–441.

Address correspondence to: Dr. Roberto Levi, Department of Pharmacology, Weill Cornell Medical College, 1300 York Avenue, New York, NY 10065. E-mail: rlevi@med.cornell.edu