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β_1-Adrenergic receptor activation decreases ANP release via cAMP-Ca^{2+} signaling in perfused beating rabbit atria

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ABSTRACT

Aims: Although a β-adrenoceptor (β-AR) blockade-induced increase in plasma atrial natriuretic peptide (ANP) levels is implicated in the therapeutic significance of β-AR antagonists, the role of β-AR in the regulation of ANP release is not clearly defined. The purpose of the present study was to define the role of β-AR subtypes and the mechanisms responsible for regulation of atrial ANP release.
Main methods: Experiments were performed in isolated perfused beating rabbit atria, including measurement of atrial contractile response, cAMP efflux, and atrial myocyte ANP release.
Key findings: β-AR activation with (−)-isoproterenol decreased ANP release concomitantly with increases in cAMP efflux concentration, atrial dynamics, stroke volume and pulse pressure in a concentration-dependent manner. The ANP response was inversely related to the change in cAMP efflux concentrations. The isoproterenol-induced decrease in ANP release was inhibited by β_1-AR blockade with CGP 20712A but not by β_2-AR blockade with ICI 118551. The isoproterenol-induced decrease in ANP release was attenuated by the L-type Ca^{2+} channel antagonist nifedipine and the cAMP-dependent protein kinase inhibitor KT5720.
Significance: These findings suggest that β_1-AR activation decreases ANP release via cAMP- and Ca^{2+}-dependent mechanisms.

Introduction

According to many if not all reports, β-adrenoceptor (β-AR) blockade increases plasma atrial natriuretic peptide (ANP) levels in patients with essential arterial hypertension (Nakaoka et al. 1987; Papadopoulos et al. 1998) or coronary artery disease (Keller et al. 1988), and in healthy volunteers (Thamsborg et al. 1987; Bouissou et al. 1989). β-AR blockade also accentuated plasma ANP levels in a population-based study (Luchner et al. 1998). Although augmentation of the natriuretic peptide system by β-AR blockade may represent a therapeutic mechanism for β-AR antagonists via guanylyl-cyclase-coupled natriuretic peptide receptor-cGMP signaling, the mechanism by which β-AR antagonists increase plasma ANP levels is poorly understood. Previous clinical findings suggest that cardiac β-AR activation inhibits ANP release.

ANP release from the atrial myocytes is primarily controlled by both the amplitude and frequency of atrial volume changes (Dietz 1984; Lang et al. 1985; Cho et al. 1991) and is modulated by various factors, including the adrenergic and muscarinic systems (Ruskoaho 1992; Xu et al. 2008). Many studies have demonstrated that β-AR activation increases ANP release from isolated atria (Schiebinger et al. 1987; Agnoletti et al. 1992). It was also shown that β-AR activation increases plasma ANP levels in anesthetized rabbits (Rankin et al. 1987). By contrast, Volpe et al. (1988) suggested that endogenous sympathetic activation can inhibit ANP release. It was also shown that β-AR activation decreases ANP release in isolated atria (Xu et al. 2008) and in cultures of atrial myocytes (Ambler and Leite 1994). Moreover, isoproterenol administration in the coronary artery decreased plasma ANP levels via β-AR stimulation in anesthetized pigs (Christensen et al. 1991). So far, our understanding of the role of β-AR in the regulation of ANP release is contradictory.

In the heart, three β-AR subtypes (β_1, β_2 and β_3) are expressed (Steinberg 1999). β_1- and β_2-AR couple through the stimulatory G protein to activate adenylyl cyclase (Xiao and Lakatta 1993; Steinberg 1999; Brodde et al. 2006).

Activation of adenylyl cyclase increases cAMP production and intracellular Ca^{2+} via both Ca^{2+} entry through L-type Ca^{2+} channels and Ca^{2+} release from the sarcoplasmic reticulum (SR). cAMP-elevating agents increase ANP release in isolated atria (Schiebinger 1989). It was also shown that cAMP is involved negatively in the regulation of ANP release in perfused atria (Cui et al. 2002) and heart (Ruskoaho et al. 1990). Intracellular Ca^{2+} is a key regulator for ANP

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release. However, the role of Ca^{2+} in the regulation of ANP release is controversial: both increases (Schiebinger 1989) and decreases (De Bold and De Bold 1989; Ruskoaho et al. 1990; Wen et al. 2000) in ANP release induced by Ca^{2+} have been reported.

The purpose of the present study was to define the role of β_1- and β_2-AR in the regulation of ANP release and the mechanisms involved (cAMP- and Ca^{2+}-dependent) in isolated perfused beating rabbit atria.

Materials and methods

Isolated perfused beating atrial reparation

New Zealand white rabbits were used to prepare isolated perfused atria as described previously (Wen et al. 2000) for atrial pacing and measurement of changes in atrial volume during contraction (stroke volume), pulse pressure, transmural extracellular fluid (ECF) translocation, cAMP efflux, and ANP secretion. In brief, the heart was removed and placed in oxygenated warm saline. The left atrium was then dissected. A calibrated transparent atrial cannula (8 cm long, 4 mm OD) containing two small catheters was inserted into the left atrium through the atrioventricular orifice. The cannula was secured by ligatures around the atrioventricular sulcus. The outer tip of the atrial cannula was open to allow for outflow from the atrium. One of the two catheters located in the atrium was for inflow. The other catheter was used to record pressure changes in the atrium. The cannulated atrium was then transferred to an organ chamber containing buffer at 36.5 °C. The pericardial space of the organ chamber was open to the air to avoid restriction of the atrial dynamics. The atrium was perfused with HEPES buffer solution by means of a peristaltic pump (1 ml/min). The composition of the buffer was as follows (mM): 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 25 $NaHCO_3$, 10.0 glucose, and 10.0 HEPES (with NaOH, pH 7.4) and 0.1% bovine serum albumin (osmolality 300.4 ± 2.6 mOsm/kg, $n = 12$). Soon after set up of the perfused atrium, transmural electrical field stimulation with a luminal electrode was started at 1.3 Hz (duration, 0.3 ms; voltage, 2 times threshold intensity, 20–30 V; 6.1-cm H_2O distension). The perfusate was prewarmed to 36.5 °C by passage through a water bath and equilibrated with oxygen by passage through silicone tubing in a gas mixing chamber. The buffer in the organ chamber was oxygenated by passing oxygen through silicone tubing coils located within the chamber. Changes in atrial stroke volume were monitored by reading the lowest level of the water column in the calibrated atrial cannula during end diastole. Atrial pulse pressure was measured via a pressure transducer connected to the intra-atrial catheter and recorded on a physiograph. To estimate transendocardial ECF translocation, transmural atrial clearance of [^{3}H] inulin was measured. Radioactivity in the atrial perfusate and pericardial buffer solution was measured on a liquid scintillation system, and the amount of ECF translocated through the atrial wall was calculated as follows: ECF translocated ($\mu l\ min^{-1}\ g^{-1}$ atrial wt) = [total radioactivity in the perfusate ($cpm\ min^{-1}$) × 1000] / [radioactivity in the pericardial reservoir ($cpm\ \mu l^{-1}$) × atrial wet wt (mg)].

Experimental protocols

The atria were perfused for 60 min to stabilize ANP secretion. [^{3}H] Inulin was introduced to the pericardial fluid 20 min before the start of sample collection (Wen et al. 2000). The perfusate was collected at 2-min intervals at 4 °C for analyses. In one series of experiments, the atria were paced at 1.3 Hz. Experiments were carried out using three groups of atria (1) to define the concentration-dependent effects of (−)-isoproterenol (ISO), (2) to determine the role of β-AR selectivity of this effect, and (3) to identify the mechanisms involved. After a control period of 48 min, the atria were perfused with various ISO concentrations (0.2 nM, group 1, $n = 7$; 2 nM, group 2, $n = 11$; 20 nM, group 3, $n = 5$; vehicle, group 14, $n = 11$). To identify the role of β-AR subtypes in the ISO-induced responses, 36-min incubation with CGP 20712A (10 nM), a β_1-AR selective inhibitor, was followed by administration of ISO (2 nM) or vehicle for 36 min in the presence of CGP 20712A (group 4, $n = 9$; CGP 20712A alone, group 5, $n = 7$). To identify the role of β_2-AR in ISO-induced responses, 36-min incubation with ICI 118551 (10 nM), a β_2-AR selective inhibitor, was followed by administration of ISO (2 nM) or vehicle for 36 min in the presence of ICI 118551 (group 6, $n = 11$; ICI 118551 alone, group 7, $n = 7$). To identify the role of Ca^{2+} entry through the sarcolemmal L-type Ca^{2+} channel in ISO-induced responses, 36-min incubation with nifedipine (Nif, 1 μM), an L-type Ca^{2+} channel selective inhibitor, was followed by administration of ISO (2 nM) or vehicle for 36 min in the presence of Nif (group 8, $n = 10$; Nif alone, group 9, $n = 8$). To identify the role of Ca^{2+} release from the SR, ryanodine (Rya, 3 μM), a SR Ca^{2+} release channel selective inhibitor, was combined with Nif. To inhibit both Ca^{2+} entry through the sarcolemmal L-type Ca^{2+} channel and Ca^{2+} release from the SR, 36-min incubation with Nif and Rya was followed by administration of ISO or vehicle for 36 min in the presence of Nif and Rya (group 10, $n = 11$; Nif plus Rya alone, group 11, $n = 9$). To analyze the role of cAMP-dependent protein kinase, 36-min incubation with a selective inhibitor, KT5720 (KT, 2 μM), was followed by administration of ISO (2 nM) or vehicle in the presence of KT5720 (group 12, $n = 7$; KT alone, group 13, $n = 5$). The effects were evaluated 36 min after administration of the agent. For time-matched or modulating-agent control, vehicle was introduced and values obtained during periods corresponding to control and experimental observations were compared.

Radioimmunoassay of ANP

Immunoreactive ANP in the perfusate was measured using a specific radioimmunoassay as described previously (Wen et al. 2000). The secreted amount of immunoreactive ANP was expressed as nanograms of ANP per minute per gram of atrial tissue. The molar concentration of immunoreactive ANP in terms of ECF translocation reflects the concentration of ANP in the interstitial space of the atrium, and therefore indicates the rate of myocyte release of ANP into the surrounding paracellular space (Wen et al. 2000). This was calculated as ANP released (in μM) = immunoreactive ANP ($pg\ min^{-1}\ g^{-1}$)/ECF translocated ($\mu l\ min^{-1}\ g^{-1}$) 3063 [molecular weight of ANP-(1–28)]. Most of the ANP secreted is processed ANP (Wen et al. 2000).

Radioimmunoassay of cAMP

cAMP was measured by equilibrated radioimmunoassay as described previously (Cui et al. 2002). In brief, standards and samples were taken up in a final volume of 100 μl of 50 mM sodium acetate buffer (pH 4.8) containing theophylline (8 mM), and then 100 μl of diluted cAMP antiserum and iodinated 2′-O-monosuccinyl-adenosine 3′,5′-cyclic monophosphate tyrosyl methyl ester were added and incubated for 24 h at 4 °C. For the acetylation reaction, 5 μl of a mixture of acetic anhydride and triethylamine was added to the assay tube before addition of antiserum and tracer. The cAMP efflux was expressed as pmol cAMP $min^{-1}\ g^{-1}$ atrial tissue. The molar cAMP efflux concentration in terms of ECF translocation reflects the concentration of cAMP in the interstitial space of the atrium. It was calculated as cAMP efflux concentration (in μM) = cAMP ($pmol\ min^{-1}\ g^{-1}$)/ECF translocated ($\mu l\ min^{-1}\ g^{-1}$). For preparation of perfusates, 100 μl of the perfusate was treated with trichloroacetic acid to a final concentration 6% for 15 min at room temperature and then centrifuged at 4 °C. The supernatant was transferred to a polypropylene tube, extracted with water-saturated ether, and then dried using a SpeedVac concentrator (Savant, Farmingdale, NY, USA). The dried sample was resuspended in sodium acetate buffer.