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Original article

Modulation of canine cardiac sodium current by Apelin

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ABSTRACT

Apelin, a ligand of the G protein-coupled putative angiotensin II-like receptor (APJ-R), exerts strong vasodilating, cardiac inotropic and chronotropic actions. Its expression is highly up-regulated during heart failure. Apelin also increases cardiac conduction speed and excitability. While our knowledge of apelin cardiovascular actions is growing, our understanding of the physiological mechanisms behind the cardiac effects remains limited. We tested the effects of apelin on the cardiac sodium current (I_{Na}) using patch clamp technique on cardiac myocytes acutely dissociated from dog ventricle. We found that apelin-13 and apelin-17 increased peak I_{Na} by 39% and 61% and shifted its mid-activation potential by -6.8 ± 0.6 mV and -17 ± 1 mV respectively thus increasing channel opening at negative voltage. Apelin also slowed I_{Na} recovery from inactivation. The effects of apelin on I_{Na} amplitude were linked to activation of protein kinase C. Apelin also increased I_{Na} "window" current by up to 600% suggesting that changes in intracellular sodium may contribute to the apelin inotropic effects. Our results reveal for the first time the effects of apelin on I_{Na}. These effects are likely to modulate cardiac conduction and excitability and may have beneficial antiarrhythmic action in sodium chanelopathies such as Brugada Syndrome where I_{Na} amplitude is reduced.

1. Introduction

Apelin, an endogenous peptide, modulates vascular tonus. The active peptides; apelin-36 (A-36), apelin-17 (A-17), and apelin-13 (A-13) results from cleavage of preproapelin. Apelin is degraded by the angiotensin conversion enzyme 2 (ACE2) [1].

It binds to the angiotensin-like putative receptor (APJ-R) [2] with affinities of 0.37 nM, 2.5 nM and 20 nM for A-13, A-17 and A-36 respectively [3]. APJ-R and cardiac sodium channels [4,5] are located at the Z-lines and intercalated discs in myocytes [6] which are key areas for conduction and contractility. Because the inhibitory effect of apelin on cAMP production is abolished by pertussis toxin, APJ-R is thought to be coupled to a G_i protein [2]. Apelin [7–9] may also modulate protein kinase A and C activity.

APJ-R is found in the cardiovascular system of many species including human [10–15]. Apelin has strong inotropic effects in isolated rat heart [9]. It is one of the strongest endogenous positive inotropic agent identified so far, yielding above 70% of the increase force observed with isoproterenol. The effects at the cardiac level are both direct (i.e. increased contractile force) and indirect (i.e. vascular) [9,16–18]. Its effects are similar to phosphodiesterase III inhibitors which exhibit cAMP- but Ca^{2+}-ATPase-dependent inotropic and vasodilating effects.

Apelin has no effect on potassium and calcium currents (I_{to}, $I_{K,sus}$, and I_{Ca}) and its ability to increase contraction is related to activation of phospholipase C (PLC) and protein kinase C (PKC) [9,19]. The sodium-hydrogen (NHE) and sodium–calcium exchangers (NCX) seem to contribute to the apelin-driven inotropic action by regulating calcium homeostasis and intracellular pH. Whether apelin directly increases intracellular calcium $[Ca^{2+}]_i$ or sensitizes myofilaments to calcium remains controversial [6,19,20].

Because apelin activates NHE, it was proposed that changes in cytosolic pH increases myofilaments sensitivity for calcium[6]. However, Dai et al. [20] found no effect of apelin on the myofilaments but demonstrated an increased systolic $[Ca2^{+}]_i$ in failing cardiac myocytes. Similarly, Wang et al. [19] reported an increase in systolic $[Ca^{2+}]_i$ but a decrease in diastolic $[Ca^{2+}]_i$ via a PKC-dependent mechanism. Apelin also increases the activity of NCX and the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) [19] via unknown mechanisms. Changes in I_{Na} amplitude and kinetics may modulate intracellular Na^{+} concentrations and the turnover of NCX to increase

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intracellular calcium. Thus, modulation of I_{Na} by Apelin could play a role in its inotropic effect.

Apelin increases the frequency of spontaneous activation and the speed of conduction [6,16]. We therefore tested if these changes in cardiac excitability were linked to modulation of I_{Na} in cardiomyocytes freshly isolated from dog left ventricle. Our results show that A-13 and A17 increase I_{Na} by 39% and 61% and shift its mid-activation potential by −6.8 mV and −8.6 mV, respectively. This resulted in a significant increase in I_{Na} window current. Neither A-13 nor A-17 modified the steady state availability (inactivation, h_∞) of the sodium channels. Recovery from inactivation was slowed by apelin and activation of PKC but not PKA was involved in the modulation of I_{Na}. Our results suggest that the apelin-induced increase in excitability is due to activation of I_{Na} at more negative potentials and that a larger window current may contribute to its inotropic effect.

2. Materials and methods

2.1. Cell dissociation

All methods and care of the dogs conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by the institutional animal ethics review committee of the University de Sherbrooke. Myocytes were isolated by enzymatic dissociation as previously described [21]. Briefly, adult mongrel dogs weighing between 30 and 35 kg were sedated with acepromazine (Atravet, 0.25 mg/kg I.M.), a phenothiazine tranquilizer, for 15 min, and then anaesthetized with heparin (5000 U) and sodium pentobarbital (25 mg/kg I.V) and their hearts quickly removed and placed in Gerrit Isenberg's Kraft-Bruhe solution. A wedge consisting of the left ventricular free wall supplied by the left anterior descending coronary artery was excised. The coronary artery was cannulated and flushed for 5 min at a rate of 5 ml/min with Ca-free Tyrode solution supplemented with EGTA 2 mM and 0.1% BSA. Perfusion was then switched to Tyrode solution at 33 °C containing 0.1 mM Ca^{2+} and 230 U/ml collagenase (CLS 2, Worthington, Freehold, NJ) and recirculated for 25–45 min. Dissociated cells were stored in Gerrit Isenberg's Kraft-Bruhe solution (in mM): 100 Potassium glutamate, 10 Potassium aspartate, 25 KCl, 10 KH2PO4, 2 $MgSO_4$, 20 Taurine, 5 Creatine, 0.5 EGTA, 20 Glucose, 10 HEPES, 2% BSA, supplemented with 0.2 mM $CaCl_2$.

2.2. Electrophysiology

2.2.1. Patch clamp

Myocytes were placed in a chamber mounted on the stage of an inverted microscope (Nikon Diaphot, Tokyo, Japan) and superfused with a low sodium solution containing (mM): 120 Choline-Cl, 5 NaCl, 5 NaOH, 2.8 Na-Acetate, 4 KOH, 0.5 $CaCl_2$, 1.5 $MgCl_2$, 10 HEPES, 10 Glucose (pH 7.4 with NaOH) to insure adequate voltage control. Tetraethylammonium chloride (5 mM) was added to the external solution to block TEA-sensitive native potassium currents and $CoCl_2$ (1 mM), 4-AP (2 mM) and $BaCl_2$ (0.5 mM) were used to block I_{CaL}, I_{To} and I_{K1} currents, respectively. Membrane currents were measured in the whole-cell configuration of the patch-clamp technique as previously described [22,23]. All recordings were made at room temperature (22 °C) using an Axopatch 200B amplifier (Axon instruments, Union City CA). Patch pipettes pulled from Corning 7052 glass (Model PP-89, Narashige, Japan) had resistance between 1.5 and 2 MΩ when filled with a solution containing (in mM) 15 NaCl, 5 KCl, 120 Cs-Aspartate, 1 $MgCl_2$, 4 Na_2-ATP, 10 EGTA and 10 HEPES (pH 7.3 with CsOH). All solutions were adjusted at 300 mOsm with sucrose. Currents were filtered with a four pole Bessel filter at 5 kHz and digitized at 10–50 kHz. Data acquisition and analysis were performed using pCLAMP programs V9.2 (Axon Instruments), EXCEL (Microsoft) and ORIGIN 6.1 (Microcal Software, Northampton, MA) softwares. Whole cell capacitance and series resistance compensation (85%) were optimized to minimize the duration of the capacitive artefact and reduce voltage errors. Only recordings from cells displaying more than six well controlled peak I_{Na} measurements between the threshold and the maximum current were analyzed. This is a well established criterion to demonstrate voltage control during I_{Na} recordings. Access resistance varied between 500 and 750 kΩ.

To avoid artefactual measurements due to time dependent voltage shifts in steady state inactivation (h_∞) in whole cell configuration, we routinely measured our first h_∞ curve after 8–10 min and at the end of the experiment i.e. roughly 20 to 30 min thereafter. Variability in $V_{0.5}$, was typically 1 to 2 mV between the 10 min and final measurements. Control data were taken in non-treated cells that were under voltage clamp for 8 min before application of Apelin.

2.2.2. Action potential measurements

Free running epicardial preparations (strips $1\times0.5\times0.1$ cm) were isolated from left ventricle (LV). The preparations, isolated using a dermatome (Davol Simon Dermatome, Cranston, RI, USA), were placed in a tissue bath (volume 5 ml, flow rate 12 ml/min) and allowed to equilibrate for at least 3 hours while superfused with oxygenated Tyrode's solution (36.5 ± 0.5 °C, pH = 7.35) and stimulated at a basic cycle length (BCL) of 500 ms using field or point stimulation (rectangular stimuli 1– to 3-ms duration, 2–3 times diastolic threshold intensity). The composition of the Tyrode's solution was (in mM): NaCl 129, KCl 4, NaH_2PO_4 0.9, $NaHCO_3$ 20, $CaCl_2$ 1.8, $MgSO_4$ 0.5, and D-glucose 5.5. Action potentials were recorded during stimulations at a cycle length of 800 ms using standard glass microelectrodes filled with 2.7 M KCl (10 to 20 MΩ DC resistance) connected to a high input-impedance amplification system (World Precision Instruments, Sarasota, FL, USA). The signals were displayed on oscilloscopes, amplified, digitized and analyzed (Spike 2, Cambridge Electronic Design, Cambridge, England). Apelin was superfused for 20 min before recordings.

2.3. Isolation of proteins

Separation of sarcolemmal and endosomal membrane fractions was performed according to established methods [24]. Briefly, fresh heart tissue was minced in a high salt solution (2 M NaCl, 20 mM HEPES, pH 7.4) and incubated for 30 min at 4 °C to depolymerize the myofilaments. Tissue was then rinsed and homogenized in a buffer containing (mM): 20 HEPES, 250 sucrose, 2 EDTA, 1 $MgCl_2$, pH 7.4. Fractionation was performed by centrifugations for 10 min at 2000*g* followed by 10 min at 5000*g*, 30 min at 200,00*g* and finally 60 min at 100,000*g*. The 5000*g* fraction containing mainly sarcolemmal membrane proteins was used for Western blots. Concentrations were determined using the BCA Protein Assay (Pierce, USA).

2.4. Immunoblots

Immunoblots were performed as previously described [23] in non-denaturing (non-reducing) conditions. Primary rabbit anti-APJ-R antibody (1:2000; Neuromics, US) was detected with an HRP-conjugated goat-anti rabbit antibody (1:3000; Bio-Rad, USA) and visualized with the Western Lightning Chemiluminescence Reagent Plus (PerkinElmer, USA). Blots were exposed to X-ray films and the intensity of each band was measured by densitometry (Quantity One software from Bio-Rad). To determine their specificity, APJ-R antibodies were pre-absorbed overnight at 4 °C against their antigen before immunoblotting or immunocytochemistry.