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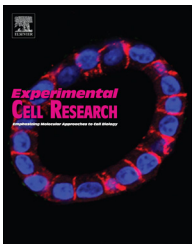

Research Article

Angiotensin (1–7) re-establishes heart cell communication previously impaired by cell swelling: Implications for myocardial ischemia

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ABSTRACT

The influence of hypertonic solution on dye coupling was investigated in cell pairs isolated from the left ventricle of adult Sprague Dawley rats.The hypertonic solution together with Lucifer Yellow CH, were dialyzed into one cell of the pair using the whole cell clamp tecnique, and the diffusion of dye in the dialyzed as well as in non-dialyzed cell, was followed by measuring the intensity of fluorescence in both cells as a function of time.The results indicated that: (1) Lucifer Yellow CH dialyzed into one cell of the pair diffuses easily into the nondialyzed cell through gap junctions; (2) the intracellular dialysis of an hypertonic solution into one cell of the pair, increases the area of the dialyzed cell and reduced the area of the non-dialyzed cell suggesting intercellular movement of water; (3) the hypertonic solution dialyzed into one cell of the pair abolished the dye coupling; (4) the gap junction permeability (*Pj*) estimated before and after administration of hypertonic solution showed an appreciably decrease of *Pj*; (5) angiotensin (1–7) (Ang (1–7) (10–9 M) administered to the bath re-established the dye coupling abolished by hypertonic solution and reduced the cell area; (6) the effect of Ang (1–7) was related to the activation of Mas receptor and was dependent on the activation of PKA. Conclusions: the reestablishment of dye coupling elicited by Ang (1–7) seen in cell pairs dialyzed with hypertonic solution, might indicate that under similar conditions like that seen during myocardial ischemia, the peptide might be of benefit preventing the impairment of cell communication and impulse propagation associated with cardiac reentrant arrhytmias.

Introduction

The preservation of normal cell volume which is of fundamental importance to cell biology, is accomplished through complex mechanisms including: (a) the Na–K–2Cl cotransport and (b) the Na/H exchanger [1]. Variations in cell volume cause important changes in cellular functions including the activation stretch-sensitive ion channels, and changes in metabolism, gene expression and protein synthesis [2,3]. Metabolites accumulate intracellularly during ischemia with consequent cell swelling caused by inward movement of to water resulting in the activation of stretch-sensitive chloride channel ($ICl_{schannel}$) which

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depolarizes the heart cell decreases the action potential duration and refractoriness [4]. Furthermore, cell swelling increases the expression of proteins like β-actin, tubulin, cyclooxygenase-2, extracellular signal-regulated kinases ERK-1 and ERK-2, JNK, the transcription factors c-Jun and c-Fos, ornithine decarboxylase, and tissue plasminogen activator [1].

Gap junctions are transmembrane channels connecting the cytoplasm of adjacent cells allowing small molecules (mol. weight <1 kDa), such as metabolites, ions and second messengers, to pass through [5]. The structural units of gap junctions are a group of membrane proteins called connexins which have four transmembrane domains and two extracellular loop domains. Three major connexins have been identified and connexin 43 (Cx43), which is mainly localized in vertebrate cardiac cells [6,7], has an important role on the spread of electrical activity throughout the heart [5] as well as on metabolic cooperation between cells [8].

Recently, evidence was presented that cell swelling induced by hypotonic stress, causes heart cell uncoupling [9] and the activation of the renin angiotensin system, which is involved in the regulation blood pressure, is also a regulator of heart cell volume. Ang II, for instance, inhibits the Na+/K+ pump and causes cell swelling [4] while the activation of ACE2 and the conversion of Ang II into Ang (1–7), counteracts many effects of Ang II [10] including the effect of Ang II on the cell volume [4]. Previous studies demonstrated that ACE2 is a major pathway for Ang (1–7) formation in the failing human heart and that the Ang (1–7) formation is significantly increased in both right and left ventricles of heart during heart failure [22]. Other studies revealed that Ang (1–7) might be protective during ischemia-reperfusion [12] reestablishing the impulse conduction by activating the sodium pump and hyperpolarizing the cell membrane [11].

It is known that the internal resistance to impulse propagation in heart fibers is mainly dependent on the gap junction resistance [5] and that a decrease in gap junction conductance reduces the conduction velocity and facilitates the generation of reentrant rhythms.

Previous observations revealed that Ang II reduces the gap junction conductance in cardiac muscle [13]. It is then important to investigate if Ang (1–7) has an opposite effect on the gap junction permeability. In the present work this problem was investigated in cell pairs of cardiomyocytes isolated from the left ventricle of normal Sprague Dawley rats.

Methods

Normal adults Sprague-Dawley rats were used. The animals were kept in the Animal House at constant temperature (24 °C) and humidity following the recommendations of NIH. Animals were kept on a normal laboratory animal diet and given tap water ad libitum. The animals were anesthetized with 43 mg/kg of ketamine plus 5 mg/kg of xylazine and the heart was removed with the animals under deep anesthesia. All animal procedures were aproved by the IACUC.

Cell isolation procedure

The heart was removed and immediately perfused with normal Krebs solution containing: (mM): NaCl—136.5; KCl—5.4; $CaCl_2$—1.8; $MgCl_2$—0.53; NaH_2PO_4—0.3; $NaHCO_3$—11.9; glucose—5.5; HEPES—5, pH adjusted to 7.3. After 20 min a Ca-free solution containing 0.4% collagenase (Worthington Biochemical Corp.) was recirculated through the heart for one hour. The collagenase solution was washed out with 100 ml of recovery solution containing (mM): taurine 10; oxalic acid 10; glutamic acid 70; KCl 25; KH_2PO_4 10; glucose 10; EGTA 0.5; pH 7.4. All solutions were oxygenated with 100% O_2. Ventricles were minced (1–2 mm thick slices) and the resulting solution was agitated gently and the suspension was filtered through a nylon gauze and the filtrate centrifuged for four minutes at 22 g. The cell pellets were then resuspended in normal Krebs solution. Suction pipettes were pulled from microhematocrit tubing by means of a controlled puller (Narashige). The pipettes that were prepared immediately before the experiments, were filled with the following solution (mM): cesium aspartate 120; NaCl 10; $MgCl_2$ 3; EGTA 10; tetraethylammonium chloride 20; Na_2ATP 5; HEPES 5; pH 7.3. Large patch electrodes (0.5–1 MΩ) were used to facilitate the diffusion of Lucifer Yellow CH between the electrode lumen and the cell interior.

Measurements of cell area

Measurements of cell length and width were made in quiescent ventricular myocytes immersed in Krebs solution using an inverted phase contrast microscope (Nikon) and a high resolution camera (Paxcam). Cell width and length were measured using ImageJ software (~150 randomly chosen cells/well, NIH, Bethesda, MD). Cell surface area (in μm^2) was calculated by multiplying cell length by cell width.

Experimental procedures

All experiments were performed in a small chamber mounted on the stage of an inverted phase-contrast microscope (Diaphot, Nikon).Ventricular cells were placed in a modified cultured dish (volume 0.75 ml) in an open-perfusion microincubator (Model PDMI-2, Medical Systems). Cells were allowed to adhere to the bottom of the chamber for 15 min and were superfused with normal Krebs solution (3 ml/min) that permits a complete change of the bath in less than 500 ms. A video system (Diaphot) made possible to inspect the cells and the pipettes throughout the experiments. The electrical measurements were carried out using the patch clamp technique in a whole cell configuration with an Axon (model 200B) patch-clamp amplifier. The leak currents were digitally subtracted by the P/N method ($n=5$–6). Experiments performed without leak subtraction indicated low and stable leak currents. Series resistance originated from the tips of the micropipettes was compensated for electronically at the beginning of the experiment.

Measurements of dye coupling

Cell pairs of ventricular myocytes were used. Patch pipettes were filled with a solution (mM): cesium aspartate 120; NaCl 10; MgCl2 3; EGTA 10; tetraethylammonium chloride 20; Na2ATP 5; HEPES 5; pH 7.3 containing 4% of Lucifer Yellow CH. The pipette was attached to one cell of the pair and after a gig ohm seal was achieved the membrane was ruptured by a brief suction allowing the dye to diffuse from the pipette into the cell. Subsequently, the dye was allowed to diffuse out of the pipette into the dialyzed cell