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Research Article

Cell-to-cell diffusion of glucose in the mammalian heart is disrupted by high glucose. Implications for the diabetic heart

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ABSTRACT

The cell-to-cell diffusion of glucose in heart cell pairs isolated from the left ventricle of adult Wistar Kyoto rats was investigated. For this, fluorescent glucose was dialyzed into one cell of the pair using the whole cell clamp technique, and its diffusion from cell-to-cell was investigated by measuring the fluorescence in the dialyzed as well as in non-dialyzed cell as a function of time. The results indicated that: 1) glucose flows easily from cell-to-cell through gap junctions; 2) high glucose solution (25 mM) disrupted chemical communication between cardiac cells and abolished the intercellular diffusion of glucose; 3) the effect of high glucose solution on the cell-to-cell diffusion of glucose was drastically reduced by Bis-1 (10^{-9} M) which is a PKC inhibitor; 4) intracellular dialysis of Ang II (100 nM) or increment of intracellular calcium concentration (10^{-8} M) also inhibited the intercellular diffusion of glucose; 5) high glucose enhances oxidative stress in heart cells; 6) calculation of gap junction permeability (P_j) (cm/s) indicated a value of $0.74 \pm 0.08 \times 10^{-4}$ cm/s (5 animals) for the controls and $0.4 \pm 0.001 \times 10^{-5}$ cm/s; $n=35$ (5 animals) ($P<0.05$) for cells incubated with high glucose solution for 24 h; 7) measurements of P_j for cell pairs treated with high glucose plus Bis-1 (10^{-9} M) revealed no significant change of P_j ($P>0.05$); 8) increase of intracellular Ca^{2+} concentration (10^{-8} M) drastically decreased P_j ($P_j=0.3 \pm 0.003 \times 10^{-5}$ cm/s). Conclusions indicate that: 1) glucose flows from cell-to-cell in the heart through gap junctions; 2) high glucose (25 mM) inhibited the intercellular diffusion of glucose—an effect significantly reduced by PKC inhibition; 3) high intracellular Ca^{2+} concentration abolished the cell-to-cell diffusion of glucose; 4) intracellular Ang II (100 nM) inhibited the intercellular diffusion of glucose indicating that intracrine Ang II, in part activated by high glucose, severely impairs the exchange of glucose between cardiac myocytes. These observations support the view that the intracrine renin angiotensin system is a modulator of chemical communication in the heart. The implications of these findings for the diabetic heart were discussed.

Introduction

Cardiac cells are communicated through gap junction channels which are composed of two oligomers. Each oligomer is a connexon which contains connexins [1]. The presence of intercellular channels makes it possible the spread of ions and electrical current from cell-to-cell which is essential for the electrical synchronization in the heart [2] and is also involved in the intercellular diffusion of

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amino acids, nucleotides, hormones and other small molecules up to 1KD contributing to the metabolic cooperation between cells [3].

Although it is known that intracellular Ca^{2+} concentration and second messengers such as cAMP and cGMP [4,5,21,16] are involved in the modulation of junctional conductance through the phosphorylation of junctional proteins [6], little is known about the functional role of metabolic cooperation between cardiac cells particularly during pathological conditions.

In the diabetic heart, the electrical coupling of cardiac cells is severely impaired [7–9,20,16] due, in part, to the PKC-dependent hyperphosphorylation of connexin 43 (Cx43) which is main connexin present in cardiac muscle [1]. A decreased expression [9], a redistribution and lateralization of Cx43 have also been described in the diabetic heart [9]. These changes impair the propagation of the electrical impulse in the diabetic heart facilitating the generation of cardiac arrhythmias.

The heart muscle is highly dependent on glucose as an energy substrate for the synthesis of ATP. Although it is known that insulin induces a rapid increase in the uptake of glucose by inducing the translocation of GLUT4 transporters from intracellular vesicles to the plasma membrane, nothing is known if cardiac cells can exchange glucose through gap junction channels. This is a fundamental aspect of heart cell biology because it involves transference of an energy substrate from cell-to-cell. It is then important to investigate if: a) glucose diffuses from cell-to-cell through gap junctions; b) hyperglycemia alters the cell-to-cell diffusion of glucose in the heart. In the present work, this problem was investigated in heart cell pairs isolated from the left ventricle of adult Wistar Kyoto rats.

Methods

Normal adult Wistar Kyoto 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 approved 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 to7.3. 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): taurine10; oxalic acid10; glutamic acid 70; KCl 25; KH_2PO_4 10; glucose 10; EGTA 0.5; pH 7.4. All solutions were oxygenated with100% O_2. Ventricles were minced (1–2 mm thick slices) and the resulting solution was agitated gently and the suspension was filtered. The filtrate was centrifuged for four minutes at 22*g*. The cell pellets were then resuspended in normal Krebs solution.

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 for15 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 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 using an Axon (model 200B) patch-clamp amplifier and Digidata 1400 (Molecular Devices, CA, USA).

To investigate the influence of high glucose on glucose transport, the concentration of glucose on the Krebs solution (Section 2.1) was increased to 25 mM and correction for hyperosmolarity, we achieved adding equivalent amounts of mannitol to normal controls.

Measurements of dye coupling

Cell pairs of ventricular myocytes were used. Suction pipettes were pulled from microhematocrit tubing by means of a controlled puller (Narashige, Japan) and filled with a solution with the following composition (mM): cesium aspartate 120; NaCl 10; $MgCl_2$ 3; EGTA 10; tetraethylammonium chloride 20; Na2ATP 5; HEPES 5; pH7.3 containing fluorescent glucose (mol weight 180 Da). In the experiments dialyzing Ca ions inside the cell EGTA was eliminated from the pipette solution. The pipette was attached to one cell of the pair, a gigohm seal was achieved and then the membrane was ruptured by a brief suction allowing the fluorescent glucose to diffuse from the pipette into the cell.

Measurements of oxidative stress

To directly monitor real time reactive oxygen/nitrogen species (ROS/RNS) a kit including an oxidative stress detection reagent (ENZO Life Sciences, Farmingdale, NY, USA) was used. Cells were exposed to high glucose (25 mM) for hours and measurements of fluorescence intensity were made before and after incubation with the oxygen/nitrogen species kit for 30 min and the green fluorescence was measured again using a wide filed fluorescence microscope equipped with standard green (490/525 nm) filter set.

Drugs

Angiotensin II, enalapril maleate and bisindolylmaleimide-1 (Bis-1) were from Sigma Chemical Co., Saint Louis, MO, USA. Fluorescent glucose was from Life Technologies (Grand Island, NY, USA).

Statistical analysis

Data are expressed as mean ± SEM. Student's *t* test was used. Differences were considered significant when $P<0.05$.

Results

To investigate the permeability of gap junction channels to glucose, the patch pipette was filled with a solution containing fluorescent glucose (4%) and the sugar was dialyzed into one cell of cell pairs