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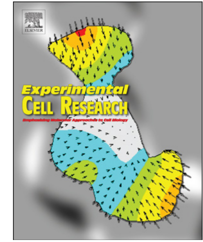

Research Article

Chemical communication between cardiac cells is disrupted by high glucose: Implications for the diabetic heart

Walmor C. De Mello*

School of Medicine, Medical Sciences Campus, UPR, San Juan, PR 00936, USA

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ABSTRACT

The influence of high glucose solution on the chemical communication between cardiac cells was investigated in cell pairs isolated from the left ventricle of adult Wistar Kyoto rats. For this, Lucifer Yellow CH was dialyzed into one cell of the pair using the whole cell clamp technique, 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) high glucose solution (25 mM) disrupted chemical communication between cardiac cells; 2) the effect of high glucose solution was reduced by Bis-1 (10^{-9} M) which is a PKC inhibitor, and by enalapril (10^{-9} M); 3) intracellular dialysis of Ang II (100 nM) also caused dye uncoupling; 4) calculation of gap junction permeability (P_j) (cm/s) indicated a value of $3 \pm 0.07 \times 10^{-5}$ cm/s; $n=32$; (6 animals) for the controls and $0.4 \pm 0.86 \times 10^{-6}$ cm/s; $n=35$ (6 animals) ($P<0.05$) for cells incubated with high glucose solution for 24 h; 5) measurements of P_j for cell pairs treated with hypertonic solution plus Bis-1 (10^{-9} M) or enalapril maleate (10^{-9} M) showed no significant change of P_j ($P>0.05$). Conclusions: high glucose (25 mM) disrupts chemical communication between cardiac cells-an effect highly dependent on PKC activation. The possible role of enhanced intracellular Ang II levels induced by high glucose on the disruption of chemical communication was discussed as well as the possible implications of these findings for the diabetic heart.

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Introduction

Cardiac cells are communicated by gap junctions making it possible the intercellular diffusion of amino acids, nucleotides and second messengers and the electrical coupling. Each gap junction channel is composed of two oligomers and each oligomer is a connexon which contains connexins. The presence of high conductance channels between cardiac cells is essential for the propagation of the electrical impulse and the synchronization of the electrical activity in the normal myocardium [1].

The junctional conductance is modulated by several factors including intracellular Ca^{2+} and second messengers such as cAMP and cGMP [1,2] which changes the junctional conductance through the phosphorylation of junctional proteins [2–4]. The activation of cAMP-dependent protein kinase (PKA) increases junctional conductance in cardiac muscle—an effect suppressed by intracellular dialysis of a PKA inhibitor [2]. Furthermore, intracellular pH and phosphorylation elicited by the oncogene v-src [5, 3] are also involved in the modulation of junctional conductance in several cell lines but in the heart, cell uncoupling

*Fax: +1 787 766 4441.
E-mail address: walmor.de-mello@upr.edu

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produced by a decrease of intracellular pH, is not within physiological levels [5].

Evidence is available that in the streptozotocin-induced diabetic rat heart, the electrical coupling of cardiac cells is severely impaired [6,7] due to the hyperphosphorylation of connexin 43 (Cx43), which is main connexin present in cardiac muscle and also to a decrease expression of Cx43 [8,6]. The hyperphosphorylation of Cx43, which is related to activation of PKC [6,9] as well as the change in redistribution and lateralization of Cx43 [10], impair the impulse propagation in the diabetic heart.

The activation of the renin angiotensin system which occurs during diabetes, contributes significantly to the deterioration of heart function in part due to the harmful effect of Ang II enhancing the oxidative stress and causing severe changes in cardiac cell communication and metabolism [11,12]. The activation of the ACE/Ang II/AT1 receptor axis, for instance, has been associated with insulin resistance [13,14] and the hyperglycemia as well as obesity, which are important components of metabolic syndrome, enhance the circulating and local cardiac Ang II levels [12].

Although evidence is available that the electrical coupling of cardiac cells is impaired in the diabetic heart [7,8,10], the question remains if hyperglycemia, by itself, is able to alter the process of cell communication and particularly the intercellular diffusion of chemical signals in the heart. In the present work, this hypothesis was investigated in cardiomyocytes 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 1 h. 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 4 min 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 with an Axon (model200B) patch-clamp amplifier. The leak currents were digitally subtracted by the P/N method (n¼ 5-6). Experiments performed without leak subtraction indicated low and stable leak currents. Series resistance originated from the tip of micropipettes was compensated electronically at the beginning of the experiment.

To investigate the influence of high glucose on the dye coupling the concentration of glucose on the Krebs solution (see above) was increased to 25 mM and to correct for hyperosmolarity, we added 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; Na_2ATP 5; HEPES 5; pH7.3 containing 4% of Lucifer Yellow CH (mol weight 457 Da). The pipette was attached to one cell of the pair and a gigohm seal was achieved. The membrane was ruptured by a brief suction allowing the dye to diffuse from the pipette into the cell.

Drugs

Angiotensin II, enalapril maleate and bisindolylmaleimide-1 (bis-1) were from Sigma Chemical Co., Saint Louis, MO,USA.

Statistical analysis

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

Results

Studies performed under control conditions, indicated that Lucifer Yellow CH (mol weight 457 Da) diffuses initially within the dialyzed cell and then into the adjacent myocyte within 30 s (Fig. 1). Since the dye is not able to cross the surface cell membrane [2], it is possible to conclude that the intercellular diffusion of the dye occurs through the gap junctions. To investigate the influence of high glucose solution on dye coupling, cell pairs were incubated in Krebs solution containing 25 mM glucose for 24 h. At the end of this time, Lucifer yellow CH was dialyzed into one cell of the pair and measurements of the intensity of fluorescence in each cell of the pair were performed, making it possible to follow the spread of the dye between the two cells. The results revealed a drastic reduction of cell communication elicited by high glucose solution as shown in Fig. 2.

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