

ON AUGMENTATION OF ADENOSINE-MEDIATED NEGATIVE DROMOTROPIC EFFECT BY K^+ RELEASED DURING MYOCARDIAL ISCHEMIA

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SUMMARY

The present study was designed to investigate mechanisms of adenosine (ADO)-mediated prolongation of conductivity through the atrioventricular (AV) node during myocardial ischemia. Using the Langendorff preparation of the guinea pig heart, we tested the hypothesis that extracellular potassium concentration elevated due to ischemia could augment ADO effect. Exposure of the heart preparation to either stop-flow or hypoxic Krebs-Henseleit solution (KH) inhibited AV node conductivity, observed as an increase in SH interval, and finally resulted in AV block. Superficial potassium concentration ($[K^+]_s$), recorded simultaneously, increased in response to each stop-flow or hypoxia. Application of 0.1 mM $BaCl_2$ markedly increased the SH interval, yet it did neither protect the heart from hypoxia-evoked AV block nor did it prevent hypoxia-induced $[K^+]_s$ elevation. Neither did perfusion of the myocardium with modified KH containing 8 mM K^+ affect the hypoxic AV block and $[K^+]_s$ increase. The hypoxic effects were not affected by adenosine A_1 agonist N6-cyclopentyl-adenosine (CPA, 30 nM). In the presence of CPA, application of high- K^+ KH, where potassium was elevated to the value of hypoxic level, did not affect the SH interval. On the other hand, adenosine deaminase (ADA, 4 U/ml) significantly attenuated the hypoxic AV block. This indicated an involvement of endogenous ADO. Yet, in the presence of both ADA and CPA, the application of the high- K^+ KH did not affect the SH interval. We concluded that increased extracellular $[K^+]_s$, elevated due to hypoxia, did not participate in the hypoxia-induced AV block mediated by ADO.

Key words: myocardial ischemia, negative dromotropic effect, adenosine, extracellular potassium

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INTRODUCTION

Modulation of atrioventricular (AV) nodal function by adenosine (ADO) has important clinical and pathophysiological significance. The perioperative course of patients undergoing heart surgery is frequently complicated by ischemia-reperfusion-induced supraventricular arrhythmia. The negative dromotropic effect of ADO underlies its therapeutic value for acute treatment of many types of supraventricular tachyarrhythmias and its role in slowing the conduction through the AV node during myocardial

ischemia (1, 2). Based on preliminary data, we hypothesized that endogenous K^+ , released during the period of ischemia, may significantly potentiate the effects of ADO by specific augmentation of the adenosine-dependent potassium current, $I_{K,ADO}$ (3, 4). The aim of the present study was to determine whether the increased K^+ concentration due to ischemia can augment the negative dromotropic effect of ADO. Therefore, the hypothesis was tested whether endogenous K^+ , released during myocardial ischemia, can potentiate the ADO-induced slowing down of AV nodal conductivity.

METHODS

Hartley guinea pigs of either sex weighing 300-400 g were anesthetized with halothane and killed by cervical dislocation. The hearts were isolated and prepared according the Langendorff method, as previously described (4, 5). The hearts were perfused at a constant flow of 4-5 ml/min/g of tissue with oxygenated Krebs-Henseleit solution (KH) containing (in mM) NaCl 117.9, KCl 3.6, CaCl₂ 2.5, MgSO₄ 1.18, KH₂PO₄ 1.2, Na₂EDTA 0.5, ascorbic acid 0.14, glucose 5.5, sodium pyruvate 2.0, and NaHCO₃ 25. KH was bubbled continuously with 95% O₂/ 5% CO₂ gaseous mixture. Hearts were paced on the low interatrial septum using an interval generator (A310 Accupulser, WPI, USA) at an atrial cycle length of 300 ms. Electrocardiograms (ECGs) were recorded using unipolar electrodes placed on the surface of the left atrium and in the vicinity of the His bundle according to a previously described method (6). Stimulus-to-His bundle (SH) interval, a measure of AV node conduction time, was obtained from the atrial and His bundle ECGs (7). Resistance of the heart coronary vessels (CR) reflecting the overall myocardial status was followed by the use of a conventional pressure transducer (Baxter Healthcare Corp., USA).

Myocardial ischemia was initiated by complete stop of the perfusion for 3 min (stop-flow). In other sets of experiments, hypoxia was applied by perfusion of the heart with hypoxic KH saturated with 5% CO₂/ 95% N₂. According to our previous findings, the concentration of oxygen in the hypoxic solution ranged between 5-10 μM, corresponding to pO₂ of 4-6 mm Hg (8). Before being subjected to 3 successive periods of ischemia or hypoxia, the hearts were allowed to equilibrate for 15 min. After each period of 3-min ischemia, they underwent reperfusion with normoxic KH for 20 minutes. The hearts were continuously immersed into warm KH in order to keep their temperature at 37.0 °C.

Superficial potassium concentration ([K⁺]_s) of the heart was recorded by the use of K⁺-selective flexible microelectrodes freshly prepared before each experiment, similarly as described previously (9). Briefly, for their preparation commercially available plastic tips for gel loading with an inner diameter of 0.1 mm were used. The narrow part of the tip was filled with a column (about 1 mm in length) of the low-impedance membrane cocktail based on the neutral K⁺-selective ion carrier valinomycin (Cocktail B 60398, Fluka). The active barrels were backfilled with 0.5 M KCl. The electrode was connected to an Axoclamp 1B amplifier (Axon Instruments Inc., Foster City, USA). Electrical signal was digitized on-line using a DigiData 1200A analog-to-digital board and stored on the hard disc of an IBM compatible Pentium computer (GP7-600 MHz, Gateway Computer, Sioux City, USA). Electrodes, placed on the heart in the area of the AV node (close to the His-bundle ECG electrode), were calibrated before and after experiments using solutions containing 1, 5, 10 and 20 mM KCl.

Values are presented as arithmetical means ± SEM. Tests of statistical significance were performed with the Student's t-test and values of p < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Exposure of the heart preparation to three consecutive applications of either stop-flow or hypoxic KH increased the SH interval

and led to AV block within 60-90 seconds. The simultaneously recorded [K⁺]_s increased in response to each stop-flow ($\Delta 1.86 \pm 0.23$ mM, n = 10) or hypoxia ($\Delta 1.24 \pm 0.16$ mM, n = 12). All the effects of stop-flow and hypoxia were reversible.

However, proper recording of [K⁺]_s became technically difficult during stop-flow since the myocardium was shrinking and the connection of the K⁺-electrode with the surface of the heart was disturbed. On pushing the electrode closely to the preparation led to KH influx into the electrode, which also interfered with proper [K⁺]_s recording.

We hypothesized that stopping the perfusion itself can cause artificial changes in recorded [K⁺]_s, which are however not related to the real increase in tissue extracellular potassium concentration. A set of experiments to prove this hypothesis was performed and the following findings were obtained. First, recorded from the hole of the plastic tube, stop-flow resulted in an increase of [K⁺]_s, even though the concentration of potassium was not altered at all. Second, the same situation occurred when a ring of the guinea-pig aorta covered the hole and [K⁺]_s recording from the surface of the aorta was performed. Moreover, artifacts by using some other electrodes, e.g. oxygen electrode, were reported as a result of changes in flow rate or uneven stirring (Manual for Using of Oxygen Monitor, Yellow Springs, USA). Finally, the increases of [K⁺]_s evoked by stop-flow and recorded under our experimental conditions were about 30-50% higher than those described in the literature on using different models of myocardial ischemia (10).

On the other hand, there were obvious advantages of modeling myocardial ischemia with the use of hypoxic KH. First, the volume of the preparation did not change during hypoxia. Thus, the [K⁺]_s recording was more stable since the K⁺-electrode was firmly sitting on the surface of the heart during the entire experiment. Indeed, values of [K⁺]_s increases due to hypoxia were found to be in good agreement with those obtained using a variety of different models of myocardial ischemia (10). Therefore, in the following sets of experiments, instead of stop-flow, we used the approach of applying hypoxic KH to model myocardial ischemia (Fig. 1).

Perfusion of the high-potassium KH containing 8 mM K⁺ through the heart did not affect the hypoxia-induced AV block. Under these conditions, application of hypoxia evoked an increase in [K⁺]_s of 1.1 ± 0.2 mM (n = 3), which did not significantly differ from that found under control conditions, i.e. during perfusion with the standard normokalemic KH (1.24 ± 0.16 mM; n = 12). The preparation responded to hypoxia during hyperkalemia by the same magnitude as when perfused with the normokalemic KH (data not shown).

Application of barium ions (Ba²⁺, 100 μM) significantly increased the SH interval ($\Delta 5.7 \pm 2.4$ ms, n = 5; p < 0.05). Yet, Ba²⁺ did neither protect the heart from the hypoxia-induced AV block nor did it prevent the evoked [K⁺]_s increase ($\Delta 1.4 \pm 0.3$ mM, n = 5). In the presence of Ba²⁺, CR was increased 3-4 times (from the basal value of 18 ± 4.7 up to 69 ± 7.0 ; p < 0.001; n = 5) and was not affected by hypoxia at all (data not shown). However, post-hypoxic recovery of heart functions, in terms of normalizing the SH interval, [K⁺]_s and CR, did not occur as long as Ba²⁺ ions were present in the perfusion solution. This is consistent with the finding that acute barium administration caused elevation of blood pressure, and also that barium affected K⁺ channels in the coronary arteries (11). Yet, underlying mechanisms have not yet been fully elucidated.

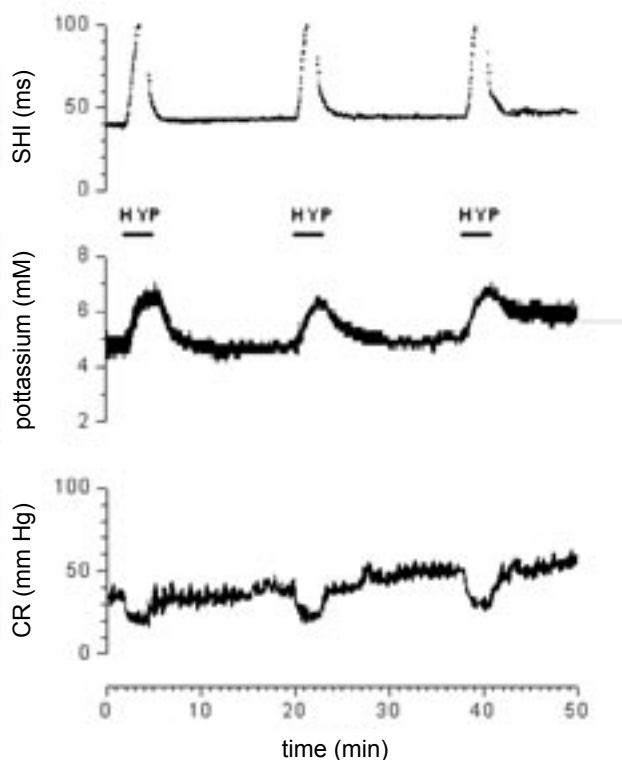


Fig. 1. Effect of hypoxia on atrial-ventricular node conduction, extracellular potassium concentration and coronary resistance of the isolated guinea-pig heart. Preparations were perfused under constant flow with Krebs-Henseleit solution (KH). Hypoxia (HYP) was applied as indicated by perfusing the heart with the hypoxic KH, saturated with 5% CO₂/95% N₂. Upper panel: stimulus-to-His bundle interval (SHI) taken from the His-bundle electrocardiogram recorded from His bundle region; middle panel: superficial K⁺ concentration recorded by the use of valinomycin electrode placed in the vicinity of the atrial-ventricular node; lower panel: resistance of coronary vessels (CR) recorded by the use of pressure transducer. Traces are representatives of recordings from one heart out of twelve.

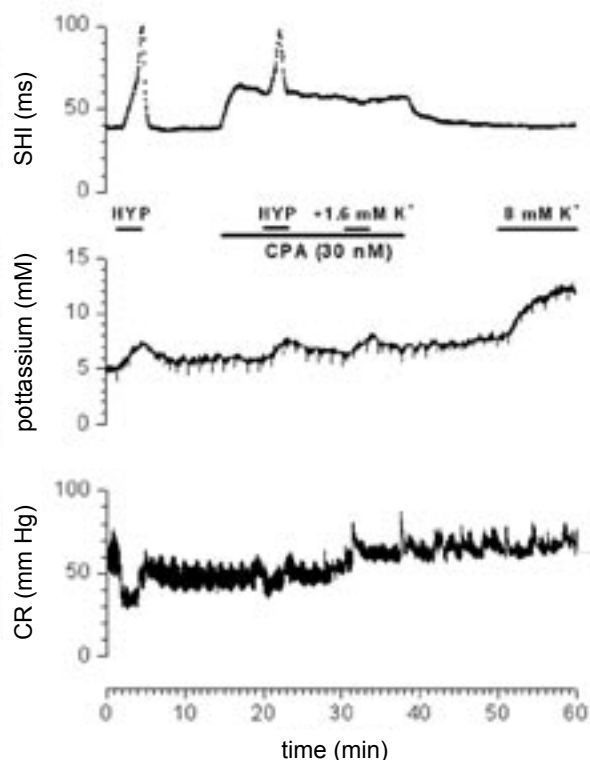


Fig. 2. Effect of adenosine A1 agonist on hypoxia- and hyperkalemia-induced changes in atrial-ventricular node conduction, extracellular potassium concentration and coronary resistance of the isolated guinea-pig heart.

Experimental conditions were analogical as described in the legend of Fig. 1. Nonhydrolyzable adenosine A1 agonist N6-cyclopentyladenosine (CPA) was applied as indicated. The extent of hyperkalemia was derived from the increase of potassium concentration observed during hypoxia (i.e. +1.6 mM). Description of panels are the same as in Fig. 1. Representatives traces of one heart out of three.

The non-hydrolyzable agonist of adenosine A1 receptors, N6-cyclopentyladenosine (CPA, 30 nM), increased the SH interval by about 20 ms. However, CPA did not affect the hypoxia-induced AV block and the evoked [K⁺]_s increase ($\Delta 1.3 \pm 0.2$ mM, n = 4).

In the presence of CPA, application of the high-K⁺ KH, on increasing the potassium level by the amount recorded during hypoxia (i.e. an increase of 1.6 mM, yielding final [K⁺] of 6.4 mM), did not affect the SH interval (n = 3) (Fig. 2).

Addition of adenosine deaminase (ADA, 4 U/ml), metabolizing endogenous adenosine, into KH significantly inhibited development of hypoxia-induced AV block (n = 4). On the other hand, changes in [K⁺]_s and CR evoked by hypoxia were not significantly affected.

Finally, CPA (30 nM) and ADA (4 U/ml) applied simultaneously inhibited development of AV block to the extent obtained by ADA alone. Yet, increasing [K⁺] in KH to the level recorded during hypoxia did not affect the SH interval in the presence of CPA and ADA at all. Therefore we concluded that the increased extracellular [K⁺], elevated due to hypoxia, did not participate in the hypoxia-induced AV block mediated by ADO.

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