Changes of β_2-adrenergic stimulation induced by hyperosmosis in human atrium

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Abstract

Purpose: The purpose of the present study was to determine whether extracellular osmotic pressure modulates β_2-adrenergic stimulation of the contraction force and L-type Ca^{2+} current in human atrial myocytes.

Material and methods: Experiments were performed on human atrial trabeculae and myocytes isolated from the right atrium. The concentration dependent effect of salbutamol (*SAL*), a β_2-adrenoreceptor agonist, on peak tension (*P*) and L-type calcium current (I_{CaL}) under isoosmolar (345 mOsm) and hyperosmolar (405 or 525 mOsm was achieved by adding of mannitol) conditions was studied.

Results: Salbutamol (10 nmol/L - 10 μmol/L) added to the control solution increased *P* by 180.6±45.8 % over control with a half-stimulation constant EC_{50} = 27±6 nmol/L. Under isoosmolar conditions *SAL* ($0.1 \div 10^3$ nmol/L) increased I_{CaL} by 182.3±19.8% over control with an EC_{50} 2.9±0.9 nmol/L. In hyperosmolar solutions the same concentrations of *SAL* increased *P* and I_{CaL} by 57.2±12.6% and 217.2±70.5% over control with EC_{50}=640±260 nmol/L and 12±5 nmol/L respectively.

Conclusions: These results indicated that hyperosmolarity reduced the effect of β_2-adrenergic stimulation, i.e. the dose-response curve of salbutamol on L-type calcium current was shifted to the higher concentration range and maximal increase in contraction force was diminished in human atrial cells.

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Introduction

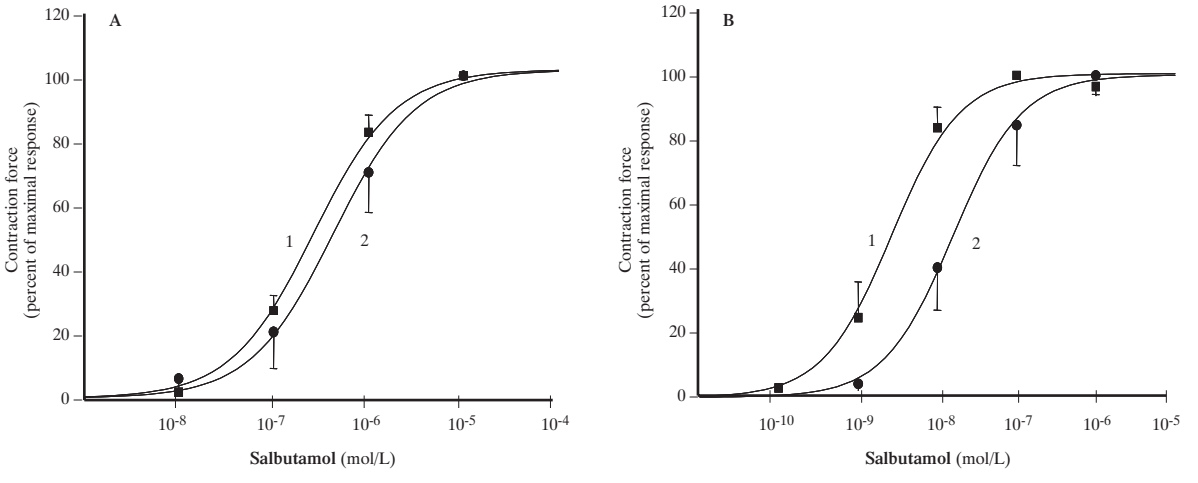
The β-adrenergic receptor system plays a major role in heart failure. β-adrenoreceptors (β-ARs) exist in the heart of various animal species, including man, and relative amount of each receptor subtypes may differ significantly depending on the cardiac tissue, the animal species, the pathophysiological state (many investigators demonstrated substantial loss of β_1-ARs but not β_2-ARs in failing human hearts) and the age. The activation of cardiac β_1- and β_2-ARs mediates inotropic, chronotropic and lusitropic effects in the heart [1]. In some pathological states such as ischemia swelling of myocardial cells is observed but during reperfusion, apoptosis or blockade of sodium pump by ouabain shrinkage of myocardial cells develops [2]. Alterations of cell volume cause the deformation of cell membranes and the underlying cytoskeletal network as well as changes of electrical activity and contractility in the heart [2,3,4]. However, it is few known about dependence of β-adrenergic stimulation of contraction and L-type calcium current in myocardial cells on extracellular osmolarity.

The purpose of the present study was to determine changes of β_2-adrenergic stimulation induced by hyperosmosis on contraction force and L-type calcium current in human atrium.

Material and methods

The experimental pieces of human atrium were obtained from the patient's hearts undergoing coronary bypass surgery in Department of Cardiosurgery of Kaunas University Hospital. The procedure was approved by the Ethical Committee of the University Hospital and conforms to the principles outlined in the Declaration of Helsinki. The standard Tyrode solution was (in mmol/L): NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.9, glucose

Figure 1. **Effect of salbutamol on the contraction force (A) and L-type calcium current (B) in human atrial preparations. A. 1 – dose-response curve in the isoosmolar solution (n=8); 2 – dose-response curve in the hyperosmolar solution (n=4). B. 1 – dose-response curve in isoosmolar solution (n=3); 2 – dose-response curve in hyperosmolar solution (n=5). The data are presented as a percentage of the maximal response to the salbutamol effect**



5, Hepes 10; pH 7.4, pO_2 (at 36-37°C) was 77-80 kPa, osmolarity 345 mOsm/L. Hyperosmosis (525 or 405 mOsm/L) was induced by adding 180 or 60 mmol/L of mannitol to the standard Tyrode solution.

The standard electromechanical activity and L-type calcium current registration methods were used [5,6]. The effect of salbutamol on contraction force (P) and L-type calcium current (I_{CaL}) were measured at iso- and hyperosmolar conditions. The dose-response curve was fitted using the Michaelis-Menten equation.

Values are means ±SE Student's t-tests for data group was used for statistical analysis. Differences were considered significant if $p<0.05$.

Results

Under isoosmolar conditions salbutamol (10 nmol/L--10 μmol/L), a selective agonist of β_2-adrenoceptors caused a potent positive inotropic effect on human atrial trabeculae. The maximal increase of contraction force (P_{max}) was 180.6±45.8% (*Fig. 1A, curve 1*). The concentration of salbutamol required for half-maximal stimulation of contraction force (EC_{50}) was 270±60 nmol/L ($p<0.05$) (n=10). Under hyperosmolar conditions (525 mOsm/L) a basal contraction force was decreased to 33.98±5.5% ($p<0.001$) versus isoosmolar conditions. In hyperosmolar solutions the dose-dependent effect of salbutamol on contraction force was: EC_{50}=640±260 nmol/L, P_{max}=57.2±12.6% (n=7) ($p<0.05$). Thus, under these conditions the efficacy of βARs stimulation was 3.14-fold lower increase in contraction force, whereas the dose-response curve was nonsignificantly shifted to the higher concentration range (*Fig. 1A, curve 2*).

A cumulative dose-response curve for the effect of salbutamol on I_{CaL} (0,1-10^3 nmol/L) in isoosmolar conditions is presented in *Fig. 1B* (*curve 1*). Salbutamol increased I_{CaL} with an EC_{50} value of 2.9±0.9 nmol/L and E_{max}=182.3±19.8% (n=3).

In hypertonic solutions dose-response curve of salbutamol on I_{CaL} is presented in *Fig. 1B*, (*curve 2*). EC_{50} and E_{max} were 12±5 nmol/L ($p<0.05$) and 217.2±70.5%, respectively ($p<0.1$) (n=5), i.e. hyperosmolarity increased E_{max} not significantly, but there was significant shift of dose-response curve to the higher concentration range of salbutamol.

Discussion

β-adrenergic stimulation of contraction force (P) and L-type calcium current (I_{CaL}) in myocardial cells is due to the stimulation of adenylate cyclase and a consequent increase in intracellular content of cAMP. cAMP activates protein kinase A resulting in phosphorylation of several proteins involved in the handling of calcium [1]. The same pathway fulfils action of salbutamol as β_2-adrenoceptor agonist.

During hypertonic cell shrinkage water is removed from cells and accumulation of intracellular ions, such as $[Na^+]_i$, $[Ca^{2+}]_i$, $[H^+]_i$ occurres [4,7,8]. In hyperosmolar solution it was shown the inhibition of the delayed rectifier K^+ current, increase of the outward-directed Na^+-Ca^{2+} exchange current, reduction I_{CaL} [4,9] and shift of pH_i to the alkaline, as well as to acidic direction [7,8]. The development of P in cardiac muscle is initiated by the binding of Ca^{2+} to troponin C. This initiation is tightly coherent with $[H^+]_i$, i.e. protons competitively inhibit the extent of Ca^{2+} binding. Negative inotropic effect of hyperosmolarity in our experiments believable was associated with intracellular acidosis [7], and with inhibition of I_{CaL} produced by markedly enhanced of $[Ca^{2+}]_i$ [9]. In shrunken myocytes, as well as in control, salbutamol caused augmentation of I_{CaL}. Less effect of salbutamol on P in hyperosmolar solutions is due to the shift of the dose-response I_{CaL} curve to the higher concentration range of the drug.

In conclusion, our experiments showed that hyperosmosis reduced the effect of β-adrenergic stimulation, i.e. substantially decreased the salbutamol stimulated contraction force and shifted the dose-response curve of salbutamol on L-type calcium current to the higher concentrations range.

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