Ph.D. Theses

Cardiac electrophysiological effects of endothelin-1 and stress-protein coinducers

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Summary

The aim of the present work was to characterize and compare the cellular cardiac effects of ET-1 on calcium and potassium currents in canine and human ventricular cardiomyocytes and to study the dose-dependent cardiac effects of bimoclomol and BRX-005 in mammalian ventricular myocardium.

The results show that ET-1 suppresses similarly the amplitude of the L-type calcium current in healthy human and canine ventricular cells. This effect of ET-1, observed in canine cells, was smaller when cAMP was elevated and was fully abolished when the PKA-system was fully activated. We conclude that the mechanism of regulation of $I_{Ca,L}$ is similar in these two species and ET-1 may exert its effect by reducing the intracellular cAMP level due to inhibition of adenilate cyclase via an ET_A receptor coupled PTX-sensitive inhibitory G-protein. ET-1 alone had no effect on the amplitude of the delayed rectifier potassium current in canine myocytes, but markedly reduced (by 80±6%) the amplitude of the rapid components of this current in human cells. These differences may be due to the contribution of different regulatory mechanisms in regulating I_K in the two species. Comparing the effects of ET-1 in canine and human ventricular myocytes, it can be concluded that canine myocytes appears to be a good model for studying the effects of ET-1 on $I_{Ca,L}$ but not on I_K in man.

Low concentrations of the heat shock protein coinducer bimoclomol and BRX-005 had comparable positive inotropic effects in working guinea pig heart, however, increased the amplitude of the intracellular calcium transients in a different degree. In contrast to bimoclomol, which caused marked increases in both systolic and diastolic calcium levels, this effect of BRX-005 was moderate. Therefore, BRX-005 can be considered as a calcium sensitizer compound. High concentrations of bimoclomol decreased the amplitude of the calcium transient due to extreme elevation of diastolic calcium. In isolated canine ventricular cells bimoclomol decreased the duration and rate of depolarization of action potentials, suppressed the inward rectifier potassium current, activated ATP-sensitive potassium channels, and induced calcium-dependent outward (chloride and/or potassium) currents. Based on the present results less side effects are anticipated with BRX-005 than bimoclomol during clinical trails.

Introduction

Endothelins are recently described vasoactive peptides released from vascular and endocardial endothelium. They represent a group of three isopeptides, from which ET-1 is an extremely potent vasoconstrictor. Endothelin receptors have been detected in various cardiac membranes

and there is now an increasing mass of evidence suggesting that ET-1 may be an important factor in cardiovascular regulation under physiological and various pathological conditions.

Effects of ET-1 on cardiac cells are controversial and strongly depend on the experimental conditions, the origin and developmental state of the preparation studied. ET-1 was shown to have positive inotropic action in several mammalian cardiac preparations, however, according to other investigators the effects of ET-1 rather resembled those of acetylcholine. ET-1 caused hyperpolarization, shortening of action potentials and reduction in heart rate due to inhibition of I_{Ca-L} and activation of K currents. Furthermore, ET-1 inhibited $I_{K(ATP)}$ in ischemic myocardium and also the protein kinase A-dependent Cl^- current in rabbit and guinea pig myocytes. These effects of ET-1 were accompanied with fall in intracellular cAMP level which was sensitive to pertussis toxin. Since the inhibitory actions of ET-1 were greatly enhanced when studied following isoproterenol treatment, the general picture emerged that the main mechanism for action of ET-1 is to inhibit the adenylate cyclase enzyme *via* the G-protein coupled ET_A receptors.

The aim of the present work was to study the effects of ET-1 on calcium and potassium currents in ventricular myocytes isolated from undiseased human and canine hearts. Due to the lack of relevant human data with endothelin and the diversity of results obtained in other mammalian cells, present results may help to elucidate the mechanism of cardiac actions of ET-1 in men.

Methods

Cell isolation

Single canine and human ventricular myocytes were obtained from hearts of adult mongrel dogs, and unused donor hearts, respectively, using the segment perfusion technique. The left anterior descending coronary artery was perfused using a Langendorff apparatus. During the initial 5 min of perfusion Ca^{2+}-free JMM solution, supplemented with taurine (2.5 g/l), pyruvic acid (175 mg/l), ribose (750 mg/l), allopurinol (13.5 mg/l) and NaH_2PO_4 (200 mg/l), was used to remove Ca^{2+} and blood from the tissue. After addition of $NaHCO_3$ (1.3 g/l), the pH of this perfusate was 7.0±0.05 when gassed with carbogene. Cell dispersion was performed for 30 min in the same solution containing also collagenase (660 mg/l, Worthington Cls-1), bovine albumine (2 g/l) and $CaCl_2$ (50 μM). During the entire isolation procedure the solutions were gassed with carbogene and the temperature was maintained at 37 ^{o}C. The cells were rod shaped and showed clear striation when the external calcium was restored. Before use, the cells were stored overnight at 14 ^{o}C in modified JMM solution (pH=7.4).

Voltage clamp

Experiments were performed in Ca^{2+}-tolerant myocytes at 37 ^{o}C. The cells were rod shaped and maintained clear cross striation following exposure to oxigenated Tyrode solution containing NaCl, 140; KCl, 5.4, $CaCl_2$, 2.5; $MgCl_2$, 1.2; Na_2HPO_4, 0.35; HEPES, 5 and glucose, 10 mM at pH of 7.4. This solution was supplemented with either 0.25 mM $CdCl_2$ or 3 mM 4-aminopyridine when potassium or calcium currents, respectively, were measured. Suction pipettes, fabricated from borosilicate glass, had tip resistance of 2 MOhm after filling with pipette solution containing K-aspartate, 100; KCl, 45; $MgCl_2$, 1; HEPES, 5; EGTA, 10; K-ATP, 3 mM, or alternatively, KCl, 110; KOH, 40; HEPES, 10; EGTA, 10; TEACl, 20; K-ATP, 3 mM, when measuring potassium or calcium currents, respectively (pH=7.2 in both cases). Membrane currents were recorded with an Axopatch-200B amplifier using the whole cell configuration of the patch clamp technique. After establishing high (1-10 GOhm) resistance seal by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by further suction or by applying 1.5 V electrical pulses for 1-5 ms. The series resistance was typically 4-8 MOhm before compensation (usually 50-80%). Experiments were discarded when the series resistance was high or substantially increasing during the measurement. Outputs from the clamp amplifier were digitized using a 333 kHz A/D converter under software control. Data were stored on video tape for later analysis. The experimental protocol for each measurement is described where pertinent in the Results section.

Measurement of intracellular Ca^{2+} transients

$[Ca^{2+}]_i$ transients were recorded in voltage clamped myocytes. Cells were loaded with the fluorescent indicator dye Fura-2 through the patch pipettes containing 100 μM K_5Fura-2 and illuminated alternatively at two wavelengths (340 and 380 nm). The excitation wavelengths were selected by the dual monochromator system of the Deltascan-1 apparatus. The emitted fluorescence was collected from a restricted area of the visual field, set by a variable slit. This area typically covered the total two-dimensional image of the cell and thus provided an optical signal that reflected the average $[Ca^{2+}]_i$. The intensity of the emitted fluorescence was measured by a photomultiplier and was not filtered. Background fluorescence was assessed in cell-attached mode prior to the establishment of the whole-cell configuration and was subtracted from the emitted fluorescence for both 340 nm and 380 nm excitations. Data acquisition was controlled by the OSCAR software using a sampling frequency of 200 Hz.

$[Ca^{2+}]_i$ is presented as a fluorescence ratio ($F_{340/380}$), corrected to the background, without calibration.

Measurement of contractility

Contractility was measured in thin trabeculae dissected from the right ventricle and individually mounted in the experimental chamber. These preparations were superfused with Tyrode solution at a flow rate of 10 ml/min. One end of each preparation was fixed to the bottom of the chamber, its opposite end was attached to the lever arm of a mechanoelectronic transducer device. The preparations were paced at 1 Hz applying extracellular stimuli of twice the diastolic threshold in amplitude, which were mediated by a pair of platinum electrodes. Before the application of ET-1 for 30 min, each preparation was allowed to equilibrate for 90 min in Tyrode solution in order to achieve steady-state conditions.

Statistics

Results are expressed as mean ± SEM values in the figures and the text. Student's t-test for paired and unpaired data was applied following ANOVA to determine statistical significance. Changes were considered significant when P was less than 0.05.

Results

Our aim was to study the effects of endothelin-1 (ET-1, 8 nmol/l) on the L-type calcium current (I_{Ca-L}) and various potassium currents (rapid component of the delayed rectifier, I_{Kr}; transient outward current, I_{to} and the inward rectifier K current, I_{K1}) in isolated human ventricular cardiomyocytes. Cells were obtained from undiseased donor hearts using collagenase digestion *via* segment perfusion technique. The whole cell configuration of the patch clamp technique was applied to measure ionic currents at 37 ^{o}C. ET-1 significantly decreased peak I_{Ca-L} from 10.2±0.6 to 6.8±0.8 pA/pF at +5 mV (66.7% of control, $P<0.05$, n=5). This reduction of peak current was accompanied with lengthening of inactivation. Voltage dependence of steady-state activation and inactivation was not altered by ET-1. $I_{Kr,}$ measured as tail current amplitudes at –40 mV, decreased from 0.31±0.02 to 0.06±0.02 pA/pF (20.3% of control, $P<0.05$, n=4) after exposure to ET-1. ET-1 failed to change the peak amplitude of I_{to}, measured at +50 mV (9.3±4.6 and 9.0±4.4 pA/pF before and after ET-1, respectively), neither steady-state I_{K1} amplitude, measured at the end of 400 ms hyperpolarization to –100 mV (3.6±1.4 and 3.7±1.4 pA/pF, n=4). Present results indicate that in undiseased human ventricular myocytes ET-1 inhibits both I_{Ca-L} and I_{Kr}, however, the degree of suppression of the two currents are different.

The effects of endothelin-1 on the L-type calcium current (I_{Ca}) and delayed rectifier potassium current (I_K) were studied in isolated canine ventricular cardiomyocytes using the whole cell configuration of the patch clamp technique. ET-1 (8 nM) was applied in three experimental arrangements: untreated cells, in the presence of 50 nM isoproterenol, and in the presence of 250 μM 8-bromo-cAMP. In untreated cells, ET-1 significantly decreased the peak amplitude of I_{Ca} by 32.3±4.8% at +5 mV (P<0.05) without changing activation or inactivation characteristics of I_{Ca}. ET-1 had no effect on the amplitude of I_K, I_{to} (transient outward current) and I_{K1} (inward rectifier K current) in untreated cells, however, the time course of recovery from inactivation of I_{to} was significantly increased by ET-1 (from 26.5±4.6 to 59.5±1.8 ms, P<0.05). Amplitude and time course of intracellular calcium transients, recorded in voltage clamped cells previously loaded with the fluorescent calcium indicator dye Fura-2, were not affected by ET-1. ET-1 had no effect on force of contraction in canine ventricular trabeculae. Isoproterenol increased the amplitude of I_{Ca} to 263±29% of control. ET-1 reduced I_{Ca} also in isoproterenol-treated cells by 17.8±2% (P<0.05), this inhibition was significantly less than obtained in untreated cells. I_K was increased by isoproterenol to 213±18% of control. This effect of isoproterenol on I_K was reduced by 31.8±4.8% if the cells were pretreated with ET-1. Similarly, in isoproterenol treated cells ET-1 decreased I_K by 16.2±1.5% (P<0.05). Maximal activation of protein kinase A (PKA) was achieved by application 8-bromo-cAMP in the pipette solution. In the presence of 8-bromo-cAMP ET-1 failed to alter I_{Ca} or I_K. It was concluded that differences in effects of ET-1 on I_{Ca} and I_K may be related to differences in cAMP-sensitivity of the currents.

Concentration-dependent effects of bimoclomol, a new HSP-coinducer, were studied on the parameters of cardiac action potential and transmembrane ion currents in enzymatically dispersed canine ventricular myocytes using conventional microelectrode and whole cell voltage clamp techniques. Bimoclomol (10-100 μM) decreased the maximum velocity of depolarization and amplitude of action potential in a concentration-dependent manner. These effects were fully reversible following a 5 min period of wash in drug-free medium. Action potential duration measured at 50% or 90% level of repolarization (APD-50 and APD-90, respectively) was markedly shortened by bimoclomol. Both APD-50 and APD-90 were decreased, but reduction of APD-50 was more pronounced than that of APD-90. The APD shortening effect of bimoclomol was significantly reduced in the presence of 20 nM charybdotoxin (inhibitor of the Ca-dependent K current) or 0.5 mM anthracene-9-carboxylic acid (inhibitor of the Ca-dependent Cl current) or 1 μM glibenclamide (activator of the ATP-sensitive K current). In contrast to control, APD-90 was lengthened by bimoclomol in the presence of anthracene-9-carboxylic acid. The APD-shortening effect of bimoclomol was also

partially antagonized by chelation of intracellular Ca^{2+} by application of the cell permeant form of BAPTA, or when using 10 mM EGTA-containing patch pipettes to record action potentials. The Vmax-depressant effect of bimoclomol was not affected by charybdotoxin, anthracene-9-carboxylic acid, glibenclamide or BAPTA-load. In voltage clamped cardiomyocytes bimoclomol (100 μM) had no effect on the peak amplitude of I_{Ca}, but decreased significantly the inactivation time constant of I_{Ca} (from 19.8±1.6 ms to 16.8±1.2 ms at 0 mV). Bimoclomol also decreased significantly the amplitude of I_{K1} (from -20.5±1.1 pA/pF to -16.6±0.8 pA/pF at -135 mV), causing reduction in slope of the negative branch of the I-V curve. At positive potentials, however, bimoclomol increased outward current. The bimoclomol induced current, therefore, was studied in the presence of $BaCl_2$, when I_{K1} current was blocked. The extra current, induced by 100 μM bimoclomol, had a reversal potential close to -90 mV. Bimoclomol (100 μM) had no effect on the amplitude or kinetic properties of the transient outward K current (I_{to}) and the delayed rectifier K current (I_K). It is concluded that bimoclomol exerts both Ca-independent (inhibition of I_{Na} and I_{K1}, activation of the ATP-sensitive K current) and Ca-dependent effects (mediated by Ca-sensitive K and Cl currents) in canine ventricular myocytes.

Concentration-dependent effects of BRX-005, the novel heat shock protein coinducer, cardioprotective and vasoprotective agent, on intracellular calcium transients and contractility were studied in Langendorff-perfused guinea pig hearts loaded with the fluorescent calcium indicator dye Fura-2. BRX-005 increased peak left ventricular pressure, the rate of force development and relaxation significantly in a concentration-dependent manner. The amplitude of $[Ca^{2+}]_i$ transients was left unaltered by the drug. In contrast to BRX-005, bimoclomol increased both contractility and the amplitude of $[Ca^{2+}]_i$ transients. In canine ventricular cardiomyocytes high concentrations of BRX-005 had no effect on depolarization, whereas bimoclomol suppressed action potential upstroke markedly. In guinea pig pulmonary artery preparations precontracted with phenylephrine, BRX-005 induced concentration-dependent relaxation. This effect of BRX-005 was independent of the integrity of endothelium indicating that vasorelaxant effect of the drug develops directly on vascular smooth muscle.

Publications

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