SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

Effects of adrenergic activation to the action potentials and ionic currents of cardiac cells

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The Examination takes place at the Department of Biophysics, Faculty of Medicine, University of Debrecen at 11am, on September 30, 2014.

Head of the **Defense Committee**:	Prof. János Szöllősi PhD, DSc
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The PhD Defense takes place at the Lecture Hall of Building A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen at 1pm, on September 30, 2014.

Introduction

Adrenergic activation of cardiac muscle is one of the most important adaptation mechanisms in the mammalian heart. These effects are mostly modulated by β adrenergic receptors and their signal transduction pathways.

Besides the well-known positive tropic changes, a number of ion currents are amplified during adrenergic stimulation, including L-type Ca^{2+} current (I_{Ca}), slow delayed rectifier K^+ current (I_{Ks}) and rapid delayed rectifierK^+ current (I_{Kr}). Little is known about the kinetics of ISO-induced amplification of I_{Kr} and I_{Ks}, however, the relative timing of activation of cardiac Ca^{2+} and K^+ currents may be especially important when discussing the mechanism of the known proarrhythmic effect of adrenergic activation.

In spite of the huge amounts of data obtained on the cardiac effects of β-adrenoceptor-mediated activation at an ion current level, there are marked interspecies differences between the effects of ISO on action potential duration including prolongation in pig, rat and guinea pig preparations, as well as shortening of action potentials in human, cat, rabbit and dog preparations.

Thus, the question arises as to whether these differences in ISO effects are a consequence of different kinetic properties of the underlying current systems or they are related to variations in action potential morphology.

In our experiments we examined the effect of β-adrenergic stimulation on the calcium and the late potassium currents. The relative contribution of I_{Kr} in the compensation of the ISO-induced augmentation of I_{Ca} has not been explored, and little was known about the kinetic properties of augmentation of I_{Kr} and I_{Ks} by ISO. Therefore, we examined and compared the timing of activation of I_{Kr}, I_{Ks} and I_{Ca}. During our experiments we examined the kinetic properties of these currents, besides the change in their amplitudes.

The effects of ISO on AP configuration of canine ventricular cardiomyocytes were studied in whole cell configuration of the patch-clamp technique

(conventional voltage clamp or action potential voltage clamp), while action potentials were recorded using sharp microelectrodes.

The role of AP morphology were examined on epi-, mid- and endocardial cellsTo study the role of AP morphology in ISO action, the midmyocardial APs were altered by blocking the I_{to}.

The changes of plateau potentials, and the duration of the action potentials were examined. The frequency-dependent properties of ISO action were studied. The β adrenergic receptor subtype involved in ISO action was identified as well.

The significance of using canine heart cells in our experimentis supported by the fact that the properties of ventricular ion currents in the human heart strongly resemble those found in canine myocardium

Aims

Our aims were

to study the effects of beta-adrenergic stimulation

- on ventricular action potential morphology and to determine its frequency dependency;
- on transmural action potential heterogeneity;

to study the role of ionic currents in beta-adrenergic response of ventricular cells;

to explore the kinetics of ion current changes during beta-adrenergic response and;

to identify the beta adrenergic receptor subtypes involved in the observed ion current and action potential changes.

Materials and methods

Isolation of single canine ventricular myocytes

Adult beagle dogs of either sex were anaesthetized, the hearts were quickly removed and placed in Tyrode solution. Single myocytes were obtained by enzymatic dispersion using the segment perfusion technique. Briefly, a wedge-shaped section of the ventricular wall supplied by the left anterior descending coronary artery was dissected, cannulated and perfused with oxygenized Tyrode solution. Perfusion was maintained until the removal of blood from the coronary system and then switched to a nominally Ca^{2+}-free Joklik solution for 5 min. This was followed by an approximately 25 min perfusion with Joklik solution supplemented with 1 mg/mL collagenase and 0.2% BSA containing 50 mM Ca^{2+}. Full transmural sections of the middle portion of the left ventricular wall were cut into small pieces and the cell suspension was washed with Joklik solution. These tissue chunks contained predominantly midmyocardial myocytes. In some experiments, cells were obtained specifically from the subepicardial or subendocardial layers of the heart by carefully slicing the left ventricular free wall. Finally, the Ca^{2+} concentration was gradually restored to 2.5 mM. The cells were stored in Minimum Essential Eagle's Medium until use.

Recording of action potentials

All electrophysiological measurements were performed at 37°C. The rod-shaped viable cells showing clear striation were sedimented in a Plexiglass chamber allowing continuous superfusion with oxygenated Tyrode solution. Transmembrane potentials were recorded using 3 M KCl-filled sharp glass microelectrodes having tip resistance between 20 and 40 MΩ. The cells were paced through the recording electrode at a steady cycle length of 1 s using 1 ms

wide rectangular current pulses with an amplitude 1.1-1.2 times that of the diastolic threshold.

Conventional voltage clamp

The cells were superfused with oxygenated Tyrode solution at 37°C. Suction pipettes, made of borosilicate glass, had tip resistances of 2 MΩ after being filled with pipette solution. Membrane currents were recorded using the whole-cell configuration of the patch clamp technique. After establishment of a high (1–10 GΩ) 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 ms. Selective blockers and voltage protocols were applied to ensure the selectivity of the studied ionic currents.

The I_{Kr} and I_{Ks} currents were characterized as a tail current amplitude determined as the difference between the peak current and the pedestal value observed following repolarization to the holding potential. I_{Ca} current amplitudes were determined as differences between peak and pedestal values. All ionic currents were normalized to cell capacitance.

Action potential voltage clamp

After establishing whole-cell configuration, action potentials were recorded in current clamp mode from the myocytes superfused with Tyrode solution. The cells were continuously paced through the recording electrode at a steady stimulation frequency of 1 Hz so as a 1–2 ms gap between the stimulus artefact and the upstroke of the action potential could occur. Ten subsequent action potentials were recorded from each cell, which were analysed online. Averaging 10 recorded action potentials, was delivered to the same cell at an identical frequency as the command voltage after the amplifier had been switched to voltage clamp mode. The current trace obtained under these conditions was a

horizontal line positioned at the zero level except for the very short segment corresponding to the action potential upstroke. The profile of the ISO-activated current was determined by subtracting the pre-drug curve from the post-drug one. This procedure resulted in a downward deflection for an inward (I_{Ca}) and an upward deflection for an outward (I_{Ks} and I_{Kr}) current, corresponding to the known fingerprints of these currents.

Statistics

Results are expressed as mean ±SEM values. Statistical significance of differences was evaluated using one-way ANOVA followed by Bonferroni's test, or by Student's T-test, according to the statistical question. Differences were considered significant when P was less than 0.05.

Results

Effects of ISO on action potential configuration

ISO induced two characteristic modifications of the canine ventricular action potential: the plateau potential, measured at half time of action potential duration, was shifted significantly towards more positive membrane potential values; and the duration of action potentials decreased significantly, that is, action potentials shortened. These changes were evident within the concentration range 10–100 nM, and were largely reversible upon washout of ISO. Since 10 nM ISO displayed reproducible and marked effects on action potential characteristics without increasing the incidence of afterdepolarizations, this concentration was used to monitor ISO effects in the presence of various ion channel blockers. Blockade of I_{Kr} by pretreatment with 1 mM E 4031 before application of ISO failed to modify the effect of 10 nM ISO. In contrast, after inhibition of I_{Ks} with 10 mM HMR 1556, the action potential duration induced by 10 nM ISO was increased, while the plateau elevation was preserved. None of these actions were elicited in the absence of functionally active L-type Ca^{2+} channels as indicated by the lack of effect of ISO effect in the presence of 5 mM nisoldipine. Since the marked interspecies differences in the ISO action may, at least partially, be related to differences in action potential configuration, the possible involvement of action potential morphology in modifying the effects of ISO was further studied by comparing the ISO-induced changes in myocytes of various origins. The effect of 10 nM ISO was also studied in myocytes digested from either the subepicardial or subendocardial layers of the middle portion of the left ventricle. The shortening effect of ISO was totally absent in endocardial cells, while little differences were observed between the epicardial and midmyocardial cells in this regard. Similar results (i.e. the lack of shortening)

were obtained when ISO was applied to myocytes that originally had a spike-and-dome action potential morphology, but their transient outward K^+ current (I_{to}) was suppressed by 1 mM 4-aminopyridine. It is important to note that ISO failed to cause action potential shortening under these conditions at any of the pacing cycle lengths applied. These results clearly demonstrate that the shape of the cardiac action potential has a critical influence on the ISO-induced changes in action potential duration, that is, the shortening can develop only in cells having a spike-and-dome action potential configuration and require both I_{Ca} and I_{Ks} to be functionally active.

Since the profile of an ion current may be quite different then studied under conventional voltage clamp and action potential voltage clamp conditions, the ISO-activated currents were studied using action potential voltage clamp. This technique enables us to record true current profiles flowing during an actual cardiac action potential. Applying of ISO induced a composite current during action potential voltage clamp measurements. The initial inward component (downward deflection) is probably mediated by I_{Ca}, while the slow rise of outward current (upward deflection) appears to be associated with I_{Ks} and I_{Kr} – as suggested by the current–voltage relationship of the ISO-induced composite current. Enhancement of these currents increased gradually when the ISO concentration was increased from 1 nM to 1 mM, and the effect of ISO was largely reversible. To develop maximal effects, 100 nM ISO was used in further action potential clamp experiments. Similar to the results obtained with ISO on action potentials, the ISOinduced composite current profiles were largely similar in the absence or presence of 1 mM E 4031. This indicates that I_{Kr}, although known to be enhanced by ISO, failed to significantly contribute to the ISO-induced current profile. When the ISO was applied after pretreatment with 10 mM HMR 1556, a potent I_{Ks} blocker, the slowly activating outward current component was almost fully abolished, which allowed us to monitor the profile of the ISO-induced inward current. 5 mM nisoldipine failed to block the ISO-

induced inward current completely, therefore 5 mM $NiCl_2$ was combined with 10 mM nisoldipine to suppress inward currents effectively. ISO failed to enhance I_{Ks} under these conditions (i.e. when the plateau of the action potential was depressed due to suppression of inward currents).

Frequency-dependent properties of the ISO-induced current

The effect of ISO on action potential configuration and the underlying ion currents is strongly frequency-dependent in guinea pig; therefore, we studied the effects of pacing rate on the ISO-induced changes in action potential duration in canine myocytes. Increasing the cycle length of stimulation resulted in an augmentation of the ISO-induced action potential shortening. When the shortening effect of 100 nM ISO was plotted against the baseline (pre-ISO) value of action potential duration at each pacing cycle length, the shortening effect of ISO appeared to be proportional to the baseline action potential duration. The magnitude of the ISO-induced inward and outward currents was also rate- dependent under action potential voltage clamp conditions. Although both components, ie. the peak inward as well as peak outward current amplitudes, increased significantly on increasing the pacing cycle length from 0.5 to 1 and 3 s, this increase was more pronounced in the case of inward currents.

Effects of ISO under conventional voltage clamp conditions

Conventional voltage clamp measurements were performed to determine the magnitude and concentration-dependence of the ISO-induced changes. Effects of cumulative increasing concentrations of ISO on I_{Ks}, I_{Kr} and I_{Ca} were determined. Superfusion of each ISO concentration lasted for 2–3 min, while washout lasted for 5–10 min. These incubation and washout periods were sufficient to develop steady-state effects and reversal of effect of each concentration of ISO. The amplitudes of I_{Ks}, I_{Kr} and I_{Ca} increased gradually with

increasing ISO concentrations. By fitting these results to the Hill equation, EC50 values of 14.5 ± 1.1, 13.7 ± 2.5 and 15.3 ± 3.5 nM were estimated with concomitant Hill coefficients close to unity. The Hill plots allowed us to determine maximal ISO effects as well. These were estimated to be 420 ± 4, 133 ± 1 and 340 ± 13% of the respective baseline values obtained for I_{Ks}, I_{Kr} and I_{Ca}, respectively.

Finally, the pharmacological suppressibility of the phosphorylated channels was compared to that of the normal, non-phosphorylated channels. In these experiments, 3 mM forskolin was used to mimic sympathetic activation. The inhibitory effects of HMR 1556, E 4031 and nisoldipine did not differ significantly between normal and phosphorylated channels.

Time-dependent effects of ISO on action potential morphology

The effect of 10 nM ISO on action potential configuration strongly depended on the transmural position of the cell. Myocytes of subepicardial and mid-myocardial origin responded to ISO with a marked shortening of action potential (both action potential duration APD_{50} and APD_{90} decreased, but APD_{20} increased). In subendocardial cells, ISO failed to reduce APD_{50} and APD_{90}, but the increase in APD_{20} could be detected. At the same time, the plateau potential was shifted significantly towards positive membrane potentials by ISO in all of the three cell types. The elevation of plateau was significant within 0.5 min after starting the ISO superfusion, while the concomitant shortening of action potentials required 1.5 and 2 min to reach the level of significance in case of APD_{90} and APD_{50}, respectively. These effects of ISO were readily reversible by a 5-min washout period with ISO-free Tyrode solution.

Time-dependent effects of ISO on the major ion currents

The shape as well as characteristic parameters of the ventricular action potential is strictly dependent of the timing and amplitude of underlying ion currents. Since ISO is known to enhance I_{Ca} and I_{Ks} strongly, and also I_{Kr} moderately, the time course of development of the ISO effect on these currents were examined under conventional voltage clamp conditions. In line with the changes in action potential configuration evoked by ISO, the ISO-induced enhancement of I_{Ca} was significant statistically within 0.5 min of exposure, while in the case of I_{Ks} and I_{Kr}, this required at least 1-minute period of time.

Contribution of β_1- and β_2-adrenergic receptors

Explanation for the observed asymmetry in the rate of the ISO-induced activation of Ca^{2+} and K^+ currents may be based on the finding that cardiac β_2-adrenergic signaling was shown to specifically stimulate a population of cardiac calcium channels by local production of cAMP, whereas β_1-adrenergic signaling stimulates calcium channels throughout the cell via a more general increase in cAMP, similarly to neuronal tissues. The question thus arises: can the two characteristic ISO-induced modifications of action potential morphology (i.e., the shortening of APD due to stimulation of I_{Ks} + I_{Kr} and the upward shift of the plateau due to enhancement of I_{Ca}) be selectively associated to different subpopulations of adrenoceptors (of course, together with the coupled specific signal transduction pathways)? This point was examined in the experiments where the contribution of β_1- and β_2-adrenergic receptors were separated using their specific blockers before applying ISO. Selective blockade of the β_2-adrenoceptors was achieved by pretreatment with 50 nM ICI 118,551, while β_1-adrenoceptors were selectively blocked by using 300 nM CGP-20712A. No change in action potential morphology was observed after pretreatment with ICI 118,551 or CGP-20712A . Blockade of the β_1-adrenoceptors converted the ISO-

induced shortening of APD to lengthening and reduced the ISO-induced elevation of the plateau potential. In contrast, inhibition of the β_2-adrenoceptors augmented the ISO-induced shortening of APD, without any effect on the plateau shift. Since ISO failed to alter any parameter of the action potential after simultaneous blockade of both receptor types, the observed effects of ISO was mediated exclusively via β_1-adrenoceptors in the presence of ICI 118,551 and exclusively via β_2-adrenoceptors in the presence of CGP-20712A. Time-dependent effects of ISO were studied also under action potential voltage clamp conditions, when the application of ISO induced a composite current: the initial inward component (downward deflection) is likely mediated by I_{Ca}, while the slow rise of outward current (upward deflection) is the sum of I_{Ks} and I_{Kr}. Enhancement of both the inward and outward current components increased progressively with time, and the effect of ISO was largely reversible. The latency of the ISO-induced enhancement was significantly shorter for the inward than for the outward current, and the initial rise of the current amplitude was also steeper in the former case. Blockade of β_1-adrenoceptors by 300 nM CGP-20712A prevented the ISO-induced enhancement of outward currents. In this case, ISO enhanced only I_{Ca}. It must be noted, however, that the amplitude of I_{Ca} induced by ISO was smaller after CGP-20712A pretreatment. Although ISO failed to activate outward currents in the presence of CGP-20712A, the latency measured for the inward current was similar in the presence or absence of CGP-20712A. The timing of changes of action potential parameters on the effect of ISO was also intimately influenced by selective β_1- or β_2-adrenergic blockade. APD_{90} was lengthened by ISO in the presence of CGP-20712A. The latency of this lengthening was similar to the latency obtained for the plateau shift either with or without CGP-20712A. Importantly, the latency for the plateau shift was not altered by CGP-20712A treatment. The opposite pattern of changes could be observed after pretreatment with ICI 118,551, where the latency for APD shortening did not change, but the latency for the plateau shift doubled.

Role of the phosphodiesterase barrier

The ISO-induced elevation of the plateau potential developed faster than the concomitant shortening of the action potential. This difference, however, was significantly smaller if the ISO exposure was performed after pretreatment with 10 μM of IBMX, the known non-selective phosphodiesterase blocker. IBMX had no effect on the latency period of the plateau shift, but accelerated the development of action potential shortening. These results suggest that the β-adrenergic signal transduction pathways governing the availability of Ca^{2+} and K^{+} channels are likely separated by at least one phosphodiesterase barrier.

Discussion

The results of our study indicate that in canine ventricular myocardium, the action potential shortening effect of isoproterenol (ISO) requires spike-and-dome action potential morphology and an upward shift in the plateau potential. The shortening is mediated by the ISO-induced activation of I_{Ks}, but is not related to I_{Kr}, and it shows reverse rate-dependent properties. ISO activates I_{Ca}, I_{Kr} and I_{Ks} with identical EC50 values. The ISO-induced changes in canine ventricular action potential morphology are also dissociated in time. The ISO-induced upward shift in the plateau (with the concomitant increase in I_{Ca}) developed earlier than the shortening of action potential duration (APD90, caused by the enhancement of I_{Ks} and I_{Kr}). Furthermore, it has also been demonstrated that activation of the β_1- and β_2-adrenergic receptors causes different patterns of action potential configuration changes, accompanied with characteristic differences in their timing.

Responses to ISO strongly vary between cardiac preparations and experimental conditions. For instance, action potential (AP) duration was found to be shortened by ISO in rabbits, dogs, cats and humans, but prolonged in pigs, rats and guinea pigs. This is absolutely in line with the interpretation of our results, that a spike and dome action potential configuration, which is a common characteristic of canine, feline and human epicardial and midmyocardial cells, is a prerequisite of the ISO induced shortening. In addition, an upward shift of the plateau potential has been observed in all mammalian preparations exposed to ISO. The increase in I_{Ca} with the concomitant positive shift in the plateau potential was also evident in the present experiments performed in epicardial or midmyocardial myocytes, while it was less pronounced, although still present, in canine endocardial cells. More importantly, this upward shift of the plateau seems to be a prerequisite of action potential shortening since action potential

duration was not modified by ISO in the presence of nisoldipine, when the plateau shift was abolished. In the presence of nisoldipine, one might expect an augmentation of the shortening effect in the absence of an enhanced I_{Ca}. However, this was not the case but, importantly, the elevation of the plateau was concomitantly abolished. Thus, the ISO-induced shortening of action potential duration required the elevation of plateau, a likely consequence of the simultaneous I_{Ca} enhancement. This positive shift in the plateau potential might stimulate/accelerate the activation of an outward current, which, at least theoretically, could be a mixture of I_{Ks} and I_{Kr} since both currents can be enhanced by ISO. Since inhibition of I_{Ks} before the application of ISO effectively prevented the shortening, while I_{Kr} blockade failed to do so, it was concluded that activation of I_{Ks} alone was the underlying mechanism for this effect of ISO. This, however, is somewhat surprising because the peak amplitude of I_{Ks} during normal repolarization of canine ventricular myocytes was estimated to be not more than 10 pA, while that of I_{Kr} was mainly 10 times higher, around 80–90 pA. Considering that I_{Ks} was augmented by ISO to 420% of its control amplitude, while I_{Kr} was enhanced only to 133% of its baseline value, the ISO-induced components of the two currents should be comparable in size. If we take into account the finding that ISO failed to shorten action potentials in the presence of nisoldipine, when both I_{Ks} and I_{Kr} could be freely stimulated by ISO, it is clear that some additional alteration beyond the direct effect of ISO on I_{Ks} and I_{Kr} is also needed to induce shortening. This crucial change is likely to be the elevation of plateau potential due to enhancement of I_{Ca}. I_{Kr} is almost fully activated at normal plateau potential; therefore, its contribution to terminal repolarization is less influenced by the plateau voltage compared to I_{Ks}, the activation of which is sharply increased by positive voltage shifts beyond this level. As a consequence, the relative contribution of I_{Ks} will be dominant under conditions where the plateau is markedly elevated. This conclusion accords with those of Varro et al. and Volders et al., who suggested

that the contribution of I_{Ks} to canine ventricular repolarization is almost negligible at rest but is an important factor following sympathetic stimulation. Our results showed that ISO shortens action potentials only in myocytes displaying a spike and dome action potential configuration. Thus, no shortening was observed in endocardial cells or in myocytes pretreated with 1 mM 4-aminopyridine. This phenomenon can be attributed to ISO-induced indirect changes in I_{Ks} kinetics. In epicardial cells, the strongly positive voltage of the late dome probably results in a larger I_{Ks} amplitude than in the case of an endocardial cell, which shows less positive membrane potential values at this time. This might explain why cardiac action potentials can be lengthened by ISO in guinea pigs, but not in canine, feline, or human epicardial tissues. The asymmetrical ISO effect (shortening of epicardial but not endocardial action potential duration) may increase the transmural dispersion through the ventricular wall during sympathetic stimulation, which may contribute to increased arrhythmia propensity under these conditions. Furthermore, since the ISO-induced shortening was increased with lengthening of the pacing cycle length, the increased dispersion will be further aggravated when the ISO challenge occurs during bradycardia, thus increasing the risk of the ‘alarm-induced’ sudden cardiac death. It must be mentioned, however, that the reverse rate-dependent nature of the ISO effect is probably not related to the known rate-dependent properties of the underlying ion currents, since in our experiments, the augmentation of I_{Ca} evoked by increasing the cycle lengths from 0.5 to 3 s was much greater than that of I_{Ks}. The explanation for this is based on the general behaviour of cardiac tissues, which produce reverse rate-dependent alterations in action potential duration in response to prolongation of baseline action potential duration. To explain the marked variability of the ISO-induced changes in various mammalian species, it was previously claimed that sympathetic stimulation modulates cardiac ion currents using different (i.e. involving more than one) signal transduction pathways. In this is true, however,

the EC_{50} values should be different, whereas we observe that ISO activated I_{Ca}, I_{Kr} and I_{Ks} with practically identical EC_{50} values and Hill coefficients; only differences in the magnitude of activation were found. This suggests that one single mechanism (probably the cAMP/PKA pathway) mediates the effects of sympathetic stimulation on I_{Ca}, I_{Kr} and I_{Ks} uniformly in canine ventricular cardiomyocytes. The involvement of stimulation of I_{Ks}, but not I_{Kr}, in the ISO-induced shortening of the canine, and probably human, ventricular action potentials has important clinical implications. Class 3 anti-arrhythmics, drugs known to block typically I_{Kr}, may carry the risk of enhanced incidence of early afterdepolarizations resulting often in torsade de pointes type arrhythmias, clearly as a consequence of the compromised repolarization reserve. Sympathetic activity is also known to increase the incidence of early afterdepolarizations, thus a combination of K^+ channel blockade with sympathetic stimulation was shown to be extremely dangerous. Application of I_{Ks} blockers as a new generation of class 3 anti-arrhythmics has been proposed due to their favourable rate-dependent properties. Extrapolation of our canine results to humans supports the conclusion that these agents may be even more dangerous in cases of an inherited or acquired form of long QT syndrome than the ‘classic’ class 3 drugs, since these patients treated with an I_{Ks} blocker will lack the ultimate mechanism of repolarization following a strong sympathetic activation. This is true in spite of the fact that I_{Ks} is thought to be an insignificant repolarizing current under baseline conditions. These considerations must be kept in mind when developing new anti-arrhythmic agents or strategies.

Activation of the β_1- and β_2-adrenergic receptors causes different patterns of action potential configuration changes, accompanied with characteristic differences in their timing. Activation of β_1-adrenoceptors (in β_2-blockade) evoked both plateau shift and APD_{90} shortening, changes considered as markers of enhancement of Ca^{2+} and K^+ currents, respectively. In contrast, isolated activation of β_2-adrenoceptors (in β_1-blockade) resulted in the prolongation of

APD_{90} (instead of shortening) combined with a less pronounced plateau shift. These changes are congruent with the enhancement of I_{Ca} without stimulation of K^+ currents. More importantly, changes induced by β_1-adrenoceptor activation developed slowly when comparing to changes initiated by selective β_2-receptor activation. Finally, all these temporal differences were abolished by inhibition of either β_1 or β_2 receptors and were significantly reduced after suspension of the phosphodiesterase barrier with IBMX. One possible explanation for the faster response of Ca^{2+} than K^+ currents to ISO might be a lower EC_{50} for the former. This possibility, however, has to be excluded, since the EC_{50} values obtained for the ISO-induced enhancement of I_{Ca}, I_{Ks}, and I_{Kr} were practically equal (15.3±3.5, 14.5±1.1 and 13.7±2.5 nM, respectively) in canine ventricular cells. These results suggest that the differences observed either in temporal manifestation of the ISO effect on various parameters of the action potential as well as in the rate of enhancement of Ca^{2+} and K^+ currents cannot be related to differences in ISO binding. Thus, alternative explanation of the present observations requires the involvement of two (at least partially independent) parallel adrenergic signal transduction pathways in canine ventricular myocytes. In adult humans and other larger mammals, β_1-adrenoceptors represent the dominant receptor population. Activation of these receptors, located in the highest density in the surface membrane, results in general effects involving activation of a variety of ion currents (including both Ca^{2+} and K^+ currents), which are accompanied by a massive increase in cytosolic cAMP concentration. On the other hand, β_2-adrenoceptors (but not the β_1 subtype) have been shown to be localized in the caveolar membrane. A full set of β_2-adrenoceptors, with the corresponding G proteins, adenylate cyclase, phosphodiesterase, protein kinase A, and Ca^{2+} channels, has been demonstrated in these rafts. Stimulation of caveolar β_2-adrenoceptors resulted in a local increase of cAMP leading to activation of I_{Ca}. The role of cardiac β_3-adrenoceptors can fully be ignored since the simultaneous blockade of β_1 and β_2 pathway eliminated all effects of ISO in

our experiments. Our results are in line with the view of dual signalization by ISO in canine ventricular myocardium. Accordingly, the majority of the ISO-induced Ca^{2+} current and the total amount of the ISO-activated K^+ current (including both I_{Ks} and I_{Kr}) are enhanced through the apparently slower β_1 pathway. When stimulated, the rapidly activating caveolar β_2 pathway adds more I_{Ca} to the system without the augmentation of K^+ currents. This may explain the temporal differences observed in the development of the ISO-induced plateau shift and APD shortening and also the conversion of APD_{90} shortening to lengthening following β_1-blockade. Congruently with our results, selective β_1-blockade eliminated the strong APD-shortening effect of ISO in rabbit ventricle and the ISO-induced enhancement of I_{Kr} in canine ventricular myocytes. Indeed, the adrenergic regulation of cardiac Ca^{2+} and K^+ channels is considered typically “different” or “independent”. In line with the concept of dual adrenergic control of I_{Ca} (together with the pure β_1-reletad regulation of K^+ currents), the time lag between the development of plateau shift and APD_{90} shortening disappeared when either the β_1- or β_2-receptor subpopulation was blocked. The significant reduction of this difference in the presence of IBMX indicates that the local cAMP gradients, built up and preserved by the phosphodiesterase barrier, may also be responsible for the asymmetry. Acceleration of the turn-on of the ISO-induced APD shortening by IBMX can be interpreted as a consequence of a cAMP load from the caveolar to the extracaveolar compartment, which is normally limited by the local phosphodiesterase barrier, although the direct effect of IBMX in this latter compartment cannot be ruled out either.

The faster activation of I_{Ca} comparing to I_{Ks} or I_{Kr} is useful when the heart must respond immediately to the demand of increased contractility. However, it may carry serious proarrhythmic risk as well, since the delay in the concomitant activation of K^+ currents increases the propensity of early afterdepolarizations, which are known to be associated with adrenergic activation. Indeed, recent

computer simulations revealed that the mismatch in timing of the ISO-induced changes in I_{Ca} and I_{Ks} may result in an increased incidence of early afterdepolarizations. In line with these predictions, the majority of sudden death cases (caused by torsades de pointes type ventricular fibrillation) occur when bradycardia is combined with strong adrenergic stimulus (e.g., sound of the alarm clock in the morning). Based on the present results, the application of selective β_2 blockers are expected to diminish the time lag between turning on the Ca^{2+} and K^+ currents and thus might decrease the risk of afterdepolarizations. This should also be considered by the clinician when setting the individual β-blocker combination for the patient.

Summary

Background and purpose

Little is known about the role of action potential morphology in the β-adrenergic receptor activation induced changes in ion currents. Therefore, the effects of the non-selective β-adrenergic receptor agonist isoproterenol (ISO) on action potential configuration, L-type Ca^{2+} current (I_{Ca}), slow component (I_{Ks}) and fast component (I_{Kr}) of the delayed rectifier K^+ current were studied and compared in a frequency-dependent manner, along with the timing of activation of each current.

Experimental approach

Whole cell configuration of the patch-clamp technique in either conventional voltage clamp or action potential voltage clamp modes were used to monitor I_{Ca}, I_{Ks}, and I_{Kr}, while action potentials were recorded using sharp microelectrodes at 37°C.

Results

In epicardial and midmyocardial cells ISO caused reversible shortening of action potentials accompanied by elevation of the plateau. These effects were associated with multifold enhancement of I_{Ca} and I_{Ks} and moderate stimulation of I_{Kr}. ISO-induced action potential shortening was absent in endocardial cells. I_{Kr} blockade failed to modify the ISO effect, while action potentials were lengthened by ISO in the presence of I_{Ks} blocker. Both action potential shortening and elevation of the plateau were prevented by pretreatment with I_{Ca} blocker. Both I_{Ca} and I_{Ks} currents increased with increasing the cycle length. The ISO-induced plateau shift and I_{Ca} increase developed earlier than the shortening of APD and stimulation of I_{Ks} and I_{Kr}. Blockade of β_1 adrenoceptors converted the ISO-induced shortening of APD to lengthening, decreased the latency of the plateau shift. In contrast, blockade of β_2 receptors augmented the APD-shortening effect and increased the latency of plateau shift. All effects of ISO

were prevented by simultaneous blockade of both receptor types. Inhibition of phosphodiesterases decreased the differences observed in the turn on of the ISO induced plateau shift and APD shortening.

Conclusion

The effect of ISO in canine ventricular cells depends critically on action potential configuration, and the ISO-induced activation of I_{Ks} – but not I_{Kr} – may be responsible for the shortening of action potentials. ISO-induced activation of I_{Ca} is turned on faster than the stimulation of I_{Ks} and I_{Kr} in canine ventricular cells due to the involvement of different adrenergic pathways and compartmentalization.

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List of publications related to the dissertation

1. **Ruzsnavszky, F.**, Hegyi, B., Kistamás, K., Váczi, K., Horváth, B., Szentandrássy, N., Bányász, T., Nánási, P.P., Magyar, J.: Asynchronous activation of calcium and potassium currents by isoproterenol in canine ventricular myocytes.
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2. Farkas, V., Szentandrássy, N., Bárándi, L., Hegyi, B., **Ruzsnavszky, F.**, Ruzsnavszky, O., Horváth, B., Bányász, T., Magyar, J., Márton, I., Nánási, P.P.: Interaction between Ca2+ channel blockers and isoproterenol on L-type Ca2+ current in canine ventricular cardiomyocytes.
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3. Szentandrássy, N., Farkas, V., Bárándi, L., Hegyi, B., **Ruzsnavszky, F.**, Horváth, B., Bányász, T., Magyar, J., Márton, I., Nánási, P.P.: Role of action potential configuration and the contribution of Ca2+ and K+ currents to isoprenaline-induced changes in canine ventricular cells.
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DOI: http://dx.doi.org/10.1111/j.1476-5381.2012.02015.x
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List of other publications

4. Hegyi, B., Komáromi, I., Kistamás, K., **Ruzsnavszky, F.**, Váczi, K., Horváth, B., Magyar, J., Bányász, T., Nánási, P.P., Szentandrássy, N.: Tetrodotoxin Blockade on Canine Cardiac L-Type Ca2+ Channels Depends on pH and Redox Potential.
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5. Jenes, Á., Szigeti, G.P., **Ruzsnavszky, F.**, Varga, A., Lőrincz, L., Csernoch, L.: Nicotine interferes with purinergic signaling in smooth muscle cells isolated from urinary bladders of patients with lower urinary tract symptoms.
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6. Kistamás, K., Szentandrássy, N., Hegyi, B., **Ruzsnavszky, F.**, Váczi, K., Bárándi, L., Horváth, B., Szebeni, A., Magyar, J., Bányász, T., Kecskeméti, V., Nánási, P.P.: Effects of pioglitazone on cardiac ion currents and action potential morphology in canine ventricular myocytes.
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Curr. Med. Chem. 18 (24), 3737-3756, 2011.
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Total IF of journals (all publications): 39.955

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