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

The species dependence of the myocardial sodium and β-adrenergic dependence of the myocardial potassium currents

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Introduction

Ion channels that coordinate the function of myocardial cells are still a widely researched topic. Although several observations have been made over the years, there are still many unanswered questions about the electrical activity of myocardial cells of the heart. One interesting fact, for example, although in ventricular myocytes of all vertebrate species, sometimes with significant differences in distribution, but the same ionic currents are essential for cellular function despite the kinetic differences. For this reason, the results obtained from the models used in cardiac electrophysiology can only be applied with appropriate weighting and therefore necessary to compare models to each other and especially to human myocardial cells. In addition, the currents creating the action potential do not only differ between species, but also within individuals, in response to certain stimuli or functional states. One of the main influencing factors of the myocardial cells is the β-adrenergic regulation. While the myocardial cells action potential and the resulting sodium, potassium, calcium and chloride currents properties have already been described in detail and we know that the myocardium as a whole is affected by the activation of the β-adrenergic pathway, but the exact background to this change remains obscure. In view of the above, I have structured my thesis around two research topics. On the one hand, I compared the properties of an important depolarizing current in cardiac myocytes, the late sodium current, in three commonly used models, dog, rabbit and guinea pig myocardial cells. In addition, I have investigated the effects of two major pathways of β-adrenergic signalling, CaMKII and PKA on two of the main repolarizing currents of the myocardial cells, the fast component of the late rectifier potassium current and the inward rectifier potassium currents.

Theoretical background

Action potential and ionic currents in left ventricular myocytes

There are a potential difference between the extra- and intracellular sides of the membrane, which varies from cell type to cell type. Also, on some cells there are ion channels that can open in response to certain stimuli, which spatially and temporally coordinated to alter the membrane potential, creating an action potential. These are called excitable cells. Such excitable cells are the muscle cells of the left ventricle of the heart.

The AP of ventricular myocardial cells can be divided into five phases (phases 0-4). The phases are formed and maintained by different ionic currents. The amplitude and shape of the ion currents are regulated by the current membrane potential and the permeability of the channels, i.e. conductance. Changes in channel conductance in abnormal conditions can change the magnitude of ionic currents and hence the shape of the AP, which can manifest itself in various cardiac arrhythmias.

At the beginning of phase 0, amswerig to the membrane depolarization, rapid time and voltage-dependent Na^+ channels are opening, establishing the early sodium current ($I_{Na,early}$) and creating the AP ascending stalk. At this time, the membrane potential rises above 0 mV, thus activating the currents responsible for further phases of the AP.

Phase 1 is a short, partial repolarization, which is caused by the two part of the transient outward current (one potassium and one chloride current) (I_{to1} and I_{to2}). The end of the phase, the L-type calcium current ($I_{Ca,L}$) is activated, which again slightly depolarizes the membrane again.

During phase 2, also called the plateau $I_{Ca,L}$ is still active and reaches its current density maximum. Although the current density of late potassium currents reaches its maximum at this time, since the equilibrium potential of potassium and calcium ions differs significantly from the membrane potential at this time, roughly equal amounts of charge flow through the membrane, so there is no pronounced de- or repolarization of the AP at this stage. In this phase, the inactivated sodium channels are in a closed state. These channels can reopen under the influence of the membrane that is now depolarized, thus forming the late sodium current ($I_{Na,late}$). At this time, the forward mode of sodium-calcium exchange current (I_{NCX}) is also active, which is the process of transporting accumulated calcium in the cell to the extracellular side.

For phase 3, the $I_{Ca,L}$ is incativated, so that the rapid and slow components of the late rectifier potassium current (I_{Kr}; I_{Ks}) have a rising activity, and with the current that otherwise responsible for the maintenance of the resting membrane potential, the rectifier potassium current (I_{K1}) repolarizes the cell membrane. In addition to the former two, the late rectifier potassium current has a third, ultra-fast component (I_{Kur}), but this
is only present in atrial myocardial cells.

Phase 4 is the electrical diastole phase, which is generated and maintained by I_{K1}, the resting membrane potential, which in myocardial cells is around -80 mV.

β-adrenergic regulation of the myocardium

The effects of stress on the body are converted to the heart tissues mainly through the activity of the sympathetic nervous system adaptation. The β-adrenergic stimulation of cardiac myocytes is mediated by G-protein coupled 7-transmembrane receptors superfamily adrenergic receptors.

Several subgroups of α1 and β receptors are found in cardiac myocytes β receptors, with a much higher proportion of β receptors than α1 receptors. β-receptors through Gs proteins increase adenylate cyclase activity in cells. They also activate the phospholipase C/phosphokinase C (PLC/PKC), CaMKII, EPAC and PI3K pathways, and affect NO synthases and TGF-β signalling.

β-receptors are involved in the cAMP/PKA pathway through through an increase in cellular adenylate cyclase activity. increases intracellular cAMP levels, which in turn activates the protein kinase A (PKA) enzyme. Activation by phosphorylation of, inter alia ryanodine receptor (RyR2) on the SR membrane of cardiac myocytes, thereby increasing the calcium content of the cell cytoplasm. It also has inhibitory effects β2-receptors, but it also inhibits K_v1.4, 4.2 and 4.3 channel proteins by phosphorylating Ito current.

The widely used β-receptor agonist isoproterenol at high concentrations (1 μM) reduces the guinea pig ventricular myocytes and canine Purkinje cells I_{K1} currents, but at low concentrations (10 nM) increased it in rodent cardiomyocytes. The role of the PKA pathway in I_{K1} current.

I_{Kr} in canine cardiac myocytes was increased by ISO and PKA activators, an effect that was observed in the presence of PKA inhibitors was abolished. Consistent with this, ISO and the adenylate cyclase activator forskolin increased I_{Kr} in guinea pig cardiac myocytes. Both these effects inhibition of both PKA and PKC, and $[Ca^{2+}]_i$ BAPTA-AM or nifedipine. In

contrast, both the ß1-receptor activation and phosphodiesterase inhibition reduced I_{Kr} PKA-dependently in guinea-pig myocytes.

Physiologically, human ventricular β-adrenergic action in cardiac myocytes, and the PLC signalling effect discussed later pathway, stimulates I_{Ks}, I_{K1} and I_{Kr} although this effect is progressively smaller in this order. It is also described that in a heart failure model, the stimulatory effect on all three potassium currents almost disappears, thereby significantly reducing the repolarization of myocardial cells reserve. It also has an effect on Nav channels, increasing the $I_{Na,late}$ produced by them.

The activation of phospholipase C (PLC) β-receptors in the cell membrane phosphoinositol bisphosphate (PIP2) into diacylglycerol (DAG) and inositol-1,3,5-triphosphate (IP3). Together, these two products activate protein kinase C (PKC) and increase $[Ca^{2+}]_i$, which also increases PKC activity. Healthy In healthy myocardium, PKC primarily regulates contractility, but its activity is increased in several myocardial diseases.

It is also important to highlight the EPAC ("Exchange Protein directly Activated by cAMP") pathway. The protein, named after the cAMP activates it. It stimulates the PKA and PKC pathways, in addition to the SRs SERCA, and RyR2 pumps, thereby fine-tuning cellular calcium homeostasis.

The role of calcium in myocardial cell function

Calcium plays a key role in the in all types of muscle tissue, including the heart muscle. The process by which electrical signal that stimulates myocardial cells is converted into a mechanical response, is called excitation-contraction coupling. The formation of AP and the contraction that occurs at the same time is derived from the extracellular space, and the $I_{Ca,L}$ and I_{NCX} reverse pathway into the cell. The remaining 70% is transported by the muscle cells sarcoplasmic reticulum (SR) of muscle cells is released by the increasing $[Ca^{2+}]_i$ via the SR ryanodine receptor (RyR2). We call this calcium induced calcium release (CICR) calcium overload, the increasing $[Ca^{2+}]_i$ inactivates $I_{Ca,L}$. However, cell relaxation requires the removal of influx or release of calcium from the cytoplasm. In this case, the calcium 70% of the calcium is re-stored in the SR via the SR calcium pump (SERCA) while the remaining 30% is pumped back into the extracellular space via the I_{NCX} forward mode and other calcium pumps.

The previously discussed sympathetic activation also increases $[Ca^{2+}]_i$. Ca^{2+} ions can mediate the effects of sympathetic activation through several pathways to the in cardiac tissues: directly, through Ca^{2+}-binding proteins such as calmodulin, and/or by complexing with other Ca^{2+}-sensitive sensitive regulatory pathways (most commonly CaMKII).

The role of the L-type calcium current in the generation of AP has been described previously in my thesis. Calcium also affects other ionic currents in cardiac myocytes. For example, Nav1.5 channels have both calcium and CaM binding sites. Some groups have found that calcium alone can alter the function of the channel, while others have concluded that only the Ca2+-CaM complex can do this. Besides direct effects, the Ca2+-CaM complex also affects Nav channels by activating CaMKII. Active CaMKII is a Ser/thr kinase that can phosphorylate Nav1.5 channels at least at three sites.

Similarly, calcium acts on the CaM-CaMKII pathway to affect potassium currents in cardiac myocytes. Whereas acutely to the β-adrenergic stimulation described earlier increases the amplitude of both IKs and IK1, chronic activity of CaMKII decreases the expression of both IKs and IK1 current-generating channels, and thus the currents themselves.

The late sodium current ($I_{Na,\ late}$)

The voltage-dependent sodium channel

Several Na_v isoforms are found in the heart of mammals, each with different voltage sensitivities, kinetics, conductivity and drug sensitivity. In the majority of heart tissue, $Na_v1.5$ is the most abundant pore-forming subunit. While this isoform is not sensitive to to the most widely effective sodium channel blocker, tetrodotoxin (TTX), other TTX-sensitive Nav channels are found in the heart. The heart $Na_v1.1$ and Nav1.6 isoforms. Associated with the pore-forming subunit of these channels proteins can also modulate the voltage-dependence of a given channel and kinetics. In the working muscle of the heart, the cell membrane depolarization of the blood cell membrane for a few milliseconds, the voltage-dependent voltage-gated sodium channels open, generating the early sodium current ($I_{Na,early}$), which forms the ascending stalk of the action potential (AP) of the working muscle cell. After opening, these sodium channels are rapidly inactivated, but the AP some of them may return to a closed state and reopen, or, in some cases, channel inactivation may not occur at all. A sodium channels are thus subject to a prolonged, late sodium current ($I_{Na.late}$) which is active during the AP plateau phase.

The mechanisms that generate $I_{Na,late}$

One of the mechanisms that generate $I_{Na,late}$ is the widely accepted theory of window-current. The basis of window-current is that the overlapping parts of the voltage-dependence ranges of the Na_v channels form a voltage window, which allows the channels to enter a

reopenable closed state from an inactive state. Under physiological conditions, in healthy myocardial cells, this phenomenon contributes minimally to $I_{Na.late}$. In certain diseases, this voltage window can be significantly altered.

The mechanism responsible for $I_{Na,late}$ to a much greater extent is based on different gating modes of voltage-dependent sodium channels. Using a series of single-channel measurements, three different modes of $Na_v1.5$ gating in cardiac ventricular tissue have been described. These are the transient (TM), burst (BM) and late scattered (LSM) modes. While $I_{Na,early}$ is largely generated by TM, the other two modes, BM and LSM, are responsible for the generation of $I_{Na.late}$. Of these, the LSM is also predominant, as the number of BM openings decreases rapidly after membrane depolarization.

In addition to the above, other factors may also play a role in the formation of $I_{Na,late.}$ These include, for example, non-equilibrium gating of the channels ("non-equilibrium gating"), where the "voltage history" of the cell membrane is induced by changes in the transitions between the states of the sodium channels. Another factor is that several Na_v isoforms are involved in the generation of the current.

The role of late sodium current in the electrical activity of the heart

Although previously low current density the role of $I_{Na,late}$ in the design of AP has been questioned, since the plateau phase is maintained by small inward and outward currents in and out, and even a small change can significantly alter the AP duration, so its importance cannot be discounted. Inhibition of current is significantly shortens the AP in the cardiac conduction system and in the ventricular muscle cells. AP voltage clamp experiments have demonstrated that current density is of a magnitude compatible with that of the major potassium currents in guinea pig and rabbit ventricular myocytes. In a previous study, our group also described that the INa.late of canine, guinea pig, and human cardiac myocytes shape in human heart muscle.

As an inward current, the $I_{Na.late}$ depolarizes the membrane, keeping the membrane potential high during the AP plateau phase phase, thereby stretching the AP itself. The longer the membrane is depolarized above +40 mV, the greater the chance that the L-type Ca channels to open or reopen. For this reason, a longer AP inevitably Ca^{2+} influx into the muscle cells.

Effect of cardiac electrical activity on late sodium current

In addition to the $I_{Na,late}$ influences action potential and electrophysiological parameters of cardiac myocytes, it the reverse is also true. As with most myocytes electrophysiological properties, $I_{Na.late}$ shows an inverse-frequency dependence, i.e. the more frequent the stimulation, the lower the current density. Thus, at higher heart rates, the $I_{Na.late}$ contributes less and less to Na^+ influx. Higher heart rate, the AP also shortens, causing INa.late to be shorter active for a shorter period of time, further reducing Na+ influx through this pathway. into cells via this route. However, the removal of Na^+ is also reduced, as the increased frequency reduces the activity of NKP, resulting in a frequency-dependent Na^+ overload on isolated cells. This phenomenon is rare, however β-adrenergic stimulation, as it increases the Na^+/K^+ pump (NKP) activity, thus stimulating sodium uptake via this pathway. removal of sodium via this route.

Fast component of the late rectifier potassium current (IKr)

The voltage-dependent potassium channels

As mentioned earlier in my thesis, the late rectifier potassium current has three components (I_{Kur}, I_{Kr} and I_{Ks}). Although the channel subunit proteins are unique to each current, thus forming the different current kinetics, their structure is the same. A channel structure is similar to the voltage-dependent sodium channel, with the difference is that while there the whole channel is made up of a single protein, here the channel is formed by four identical α-subunits forming a homotetramer. From α-subunits have six transmembrane segments and a pore-forming N- and C-terminals are located intracellularly. A voltage sensor is the S4 segment of the subunits, while the K^+ selectivity of the amino acid chain connecting the S5-S6 segments. The fast component (I_{Kr}) of the current is the ion channel $K_v11.1$ channel protein. The channel is the front end of the action potential rapid depolarization, and then rapidly inactivated. This is achieved by a followed by a slow reopening and then the channels close. In this AP section amplitude is caused by the increasing number of channels entering the This is called current accumulation.

Electrophysiological properties of IKr

The current is activated when the membrane potential becomes more positive than -40 mV. Its amplitude reaches a maximum at 0 mV, at values of membrane potential more positive than 0 mV the current density decreases. The inward rectifying property of the current is due to the previously mentioned gating kinetics of $K_v11.1$. Although the current is activated by

depolarization at the beginning of the AP, it is also inactivated almost immediately due to a positive membrane potential, so that it has no repolarizing effect during the plateau phase of the AP. However, as the membrane potential approaches 0 mV again due to the closure of the calcium channels, the channels reopen. The current is then the main driver of repolarization, responsible for initiating terminal repolarization. In its absence, the ventricular action potential is significantly stretched, providing a breeding ground for cardiac arrhythmias.

Effects modifying the operation of the current

Extracellular $[K^+]$ is an elementary determinant of current activity. An increase in $[K^+]_e$ increases the current density, while a decrease in potassium levels also inhibits the current, in addition to internalizing and degrading the IKr-generating channel. Acidosis of the body, by modifying the voltage dependence of current activation and accelerating its deactivation, also reduces IKr density. Divalent cations also inhibit the function of the current. There are several selective inhibitors of current, such as E-4031, d-Sotalol, and Dofetilide. In addition, other drugs that act on organ systems also have IKr inhibitory effects, such as the antipsychotic Haloperidol and the antiviral Dasabuvir.

Inward rectifying potassium current (I_{K1})

The inward rectifying potassium channel

The inward rectifying channels are simpler than their voltage-gated counterparts. In humans, the I_{K1} channel is the main pore-forming protein of $K_{ir}2.1$, but $K_{ir}2.2$ and $K_{ir}2.3$ subunits are also involved homo- or heteromeric forms. The α subunit consists of two transmembrane domains between which the pore-forming region is located. Four of these subunits form a functional channel. In guinea-pig ventricular myocytes, four smaller conductive and one fully open state of the channel have been described. Although the channel is neither ligand nor voltage gated, it is a rectifying characteristics of the intracellular side in a voltage- and time-dependent manner Mg^{2+} and polyamines ($spermidin^{3+}$, $spermin^{4+}$) are responsible for the binding of the outward current from -40 mV to more positive membrane potential.

Factors affecting the operation of the inward rectifier potassium current

The I_{K1} creator channel is enhanced by the extracellular [K^+] elevation, Ba^{2+}, Cs^+ and various antiarrhythmic pharmacones (e.g. amiodarone) inhibit the channel. Intracellularly in cells acidosis, oxidative stress and lysophosphatidylcholine may exert inhibitory effects.

Interspecies differences in ion currents in ventricular myocytes

From in the animal kingdom, the action potentials of cardiac myocytes are significantly differ in their morphology. These differences depend on the body size of the animal, its heart rate, but the oxygenation and temperature of the environment. Despite the huge variation between species, AP is still affected by the same voltage-gated ion channels in all mammals. AP waveforms are primarily due to differences in heart rate differences in gene expression due to differences in heart rate.

All vertebrate action potential begins with a rapid depolarization of phase 0. The phase 1 is expressed only in mammals and birds, in which case only in certain layers of the ventricular wall. The platonic phase also has a shape and size in the action potential of different species. While the plateu phase of some reptiles can last up to seconds, whereas the ventricular AP of most rodent species can last up to a rapid repolarization immediately after the AP peak. The third and fourth phases are also universal, although the resting membrane potential value also shows variation between species.

A for the null phase and myocardial propagation of membrane depolarization in the I_{Na} in all vertebrates. In the heart of most vertebrates, $Na_v1.5$ is the main pore-forming subunit, but in mammals (including humans) other subunits, mainly isoforms occurring mainly on neurons. In general, it can be said that in vertebrates studied at the same temperature myocardial cells at the same temperature showed similar I_{Na} current densities. However, there is a difference in the TTX sensitivity of sodium channels. This is mainly due to the $Na_v1.5$ gene specific mutation and the composition of the channel α-subunit.

The mammalian cardiac myocytes, the calcium induced calcium release (CICR) is essential for the mammalian muscle cell function. In turtles, and several contraction of myocardial cells is much more dependent on extracellular Ca^{2+} influx rather than CICR.

From I_{Kur} is not found in vertebrates other than mammals, and Ito, or other fast kinetic K^+ currents, despite the fact that their AP has a first phase. In some rodent species (mouse, rat, and groundhog), cells repolarization of cells is completely controlled by fast potassium currents (I_{Kur}, and I_{to}) which allows particularly high heart rates.

From I_{Ks} and I_{Kr} full activation of the channels that create them and the degree of membrane depolarisation, the number of channels may be several tens of seconds. In fish cardiac myocytes, I_{Kr} is mainly responsible for membrane repolarization and its current density is particularly high. The current activation kinetics are slower and inactivation kinetics are faster in mammalian cardiomyocytes found in mammalian cells. Among all vertebrates, the I_{Kr} is the lowest, while the data from quails suggest that birds cardiomyocytes have a significant density of IKr, which is much higher than in quails. exceeds that of many mammals. The I_{Kr} of amphibians with significant current density has long been known, but in fish ventricular myocytes it has only recently been shown that the slow component of the late rectifier potassium current also plays an important role in AP formation.

The inward rectifier potassium current (I_{K1}) is responsible for maintaining the resting membrane potential of all vertebrate cardiomyocytes. Nevertheless, it can be said that between strains, and even between species differences in current density, which may be phylogenetic and specific to the animal the life history of the individual animal. In fish, especially in physically active species, the IK1 amplitude of ventricular myocytes is markedly high.
Relatively little is known about IK1 currents in reptile and amphibian hearts. On the contrary, it can be said that the winged and mammalian cardiomyocytes have a prominent I_{K1} current, which probably because they are endothermic organisms.

All these differences that the species of animals used as myocardial cell models are the same ionic currents of the same cell types used as models is of particular importance in the results obtained in pharmacological studies. interpretation of the results obtained in pharmacological studies.

Statement of the problem and objective

Late sodium amphiphiles and conductance analysis in dog, rabbit and guinea pig models

Late sodium current has been studied in several models, including guinea pig, pig, rabbit, dog and human cardiac myocytes. These studies have largely been performed using a conventional voltage clamp technique, which has the disadvantage that the physiological shape of the current below the action potential cannot be observed. This problem can be avoided by using the action potential voltage clamp technique, but there are currently few studies on this topic, which mainly focus on ionic current recordings and do not address the conductance changes during the action potential. In a previous study, our group compared $I_{Na,late}$ current profiles of canine and human cardiac myocytes, but we did not monitor the amount of charge passing across the membrane. Thus, the aims of our current work were:

- to simultaneously study the late sodium current profile ($I_{Na,late}$) and conductance variation ($G_{Na,late}$) using APVC technique in three commonly used mammalian animal models (dog, rabbit, guinea pig)
- to investigate the shape of the action potential as an influencing factor in canine cardiomyocytes using canonical command action potentials from different species using APVC technique
- comparison of the sodium current formed by Anemone toxin II (ATX-II) with the shape and conductance changes of the native late sodium current

Outward rectifier potassium current and β-adrenergic activation of the fast component of the late rectifier potassium current by the CaMKII pathway

While it can be argued that the sympathetic stimulation affects the expression of both I_{K1} and I_{Kr} currents in ventricular myocytes, the exact way in which this is done is still in doubt. In addition, the picture is further complicated by the close relationship between the PKA and CaMKII pathways the close association between the two pathways. Thus, the aim of this part of our research was to isolate the two previously mentioned intracellular pathways of β-adrenergic activation pathways in the regulation of the two potassium currents in cardiac myocytes.

Materials and methods

Isolation of cardiomyocites

During the research work of our working group dogs, guinea pigs and rabbits. In the case of dogs, they were cultured for experimental purposes, from the left ventricle of the heart of 10-12 month old adult beagle dogs. from the isolated myocardial cells of the heart. The animals were overexposed to ketamine hydrochloride and and xylazine hydrochloride. Once we were convinced that the anaesthesia depth of anaesthesia, we opened the animal's chest and then the heart as quickly as possible. The organ was then drained of its blood was flushed with tyrode's solution to remove most of the blood. The myocardial cells from the heart using the so-called anterograde segment perfusion method. In this technique, the left first left descending coronary artery (LAD) was cannulated and the blood supply area of the vessel perfused the heart muscle using a Langendorff apparatus.

During the first phase of isolation, the first 5 min a modified JMM solution was used to remove the remaining blood and Ca^{2+} content of the heart. Afterwards, in the second phase, the previous perfusion solution was replaced with collagenase and calf albumin supplemented with JMM solution, with which we continued the perfusion for 30-45 minutes, depending on the size of the heart.

After digestion is complete, the cannulated vessel supply U-shaped incision was made in the area of the left ventricular wall, approximately the midmyocardial layer of the left ventricular vein, thus freeing the liquefied tissue, which was then treated with Ca^{2+} in the first phase of isolation. supplemented JMM solution and suspended. The last step of isolation phase of the final isolation step, this cell suspension was sedimented for 4 cycles (each cycle 9 min per cycle), then filtered with an increasing mesh density filter and washed with a JMM solution of increasing Ca^{2+} concentration. Subsequently the cells were washed with MEM solution for 2 more cycles without filtration.

After isolation, the cells were washed with the aforementioned MEM solution at 14 °C. Measurements were not performed until at least 2-3 hours after the end of isolation. after the isolation of the cells. At this time, 2/3 of the cells were rectangular, intact and sharp-edged, and with retained striations.

For guinea pigs and rabbits, the process of cell isolation was similar. The animals were first coagulated with heparin and then anesthetized with nembutal. After the onset of sufficiently deep anaesthesia the hearts of the animals were removed from their chests and held by the aorta, Langendorff by retrograde perfusion and standard enzymatic isolation was

performed. The hearts were first washed with oxygenated Tyrode's solution for 5 min. Subsequently, they were subjected to Ca^{2+} free Tyrode's solution was used for organs for 3 minutes. The left ventricle of the hearts was then dissected and the resulting pieces were subjected to the enzymatic treatment mentioned above for a further 1 hour. solution mentioned above. The extracted cells were then incubated with Tyrode solution until use.

The methods used were consistent with both the "Guide for the Care and Use of Laboratory Animals" (US NIH publication No 85-23. revised 1996) and the principles of the Declaration of Helsinki, in addition to the the experimental protocols were approved by the University of Debrecen's (No. 51-57/1997 OEJ) (9/2015/DEMÁB).

Electrophysiology

Before starting the measurements, the myocardial cells on an inverted slide with a volume of 1 ml, heated by a temperature regulator in a Plexiglas measuring bath, heated by a thermostated thermostat, which is placed in a Faraday cage with an anti-vibration table. Cells containing the bicarbonate mentioned earlier, modified Tyrode's solution, using a gravity-driven perfusion system.

The measurements are performed with a resistance of 2-3 MΩ microelectrodes (patch pipettes), which are coated with borosilicate glass capillaries using a pipette puller. To measure ion currents the internal solution of the patch pipette used for the measurements of the conductivity of the pipette pipettes were supplemented with active substances.

To carry out the measurements pipettes were pulled onto a silver chloride electrode connected to a head unit, which carries the electrical analogue signals of the cells. This head unit is a three mechanical macro- and piezoelectric transducer that can be moved in three directions. micromanipulators, which are used to to approach and touch the cells with the patch pipette.

Ion currents are measured on a MultiClamp 700B amplifier after analog-to-digital conversion using a Digidata 1440A are recorded on the computer using pClamp 10.0 software.

To start the measurements, the cells are are approached under a microscope and then touched with the tip of the patch pipette. A gentle suction is then applied, reducing the pressure in the pipette, which causes the part of the cell membrane that touches the tip of the pipette to be squeezed into the pipette. A high-resistance (1-10 GΩ) contact is then formed between the pipette between the pipette and the cell. Once this so-called "gigaseal", a sudden suction or blowing action is used to break through the cell membrane in the pipette, thus forming the patch-clamp technique whole-cell arrangement. After this, a 15 ms long, +10 mV to -10 mV to

10 mV, we determined the cellular membrane capacitance, which is proportional to the cell membrane surface area. To do this is necessary because the measured ionic currents can be used to calculate the capacitance of the cell membrane. to the membrane membrane of the cells, we can eliminate the differences due to the cell size. After determining the cell membrane capacitance, the measuring system current-clamp mode, the cells are clamped by the DS-R3 pulse generator 2 ms wide, 700 ms cycle length square-wave pulses generated by the DS-R33 pulse clamp generator were recorded. action potential (AP) of the myocardial cells. which can be inferred from the shape of the AP. Then we can start recording ion currents.

Ion current measurement with conventional voltage clamp technique

To study the β-adrenergic dependence of I_{K1}, conventional voltage clamp technique was also used. To perform this technique, the patch clamp was fully cellular arrangement, the cells were clamped for 250 ms test pulses, the I_{Kr} and I_{Ks} currents were after pharmacological inhibition. The amplitude of the test pulses ranged from -80 mV to +20 mV with a holding potential of -80 mV.

Ion current measurement with action potential voltage clamp technique (APVC)

The AP of excitable cells ion currents involved in the formation of the action potential voltage-clamp technique (action potential voltage-clamp, APVC) is the most suitable. In our measurements, to perform the APVC technique either as a voltage command or as a voltage command from a previously recorded average canonical action potential that best approximates the average parameters, or we used the cell's own action potential. Thus stimulating the cells, the measured reference current is zero when using the own AP, as it is no current from the amplifier is needed at all, the canonical action potential is close to zero, since the cells hardly need any power from the amplifier to maintain their own AP. The reference current the ion current inhibitor of interest is added to the perfusion solution to the perfusion solution to block the channel that generates the ion current. The current thus lost is called must be replaced by the amplifier in order to maintain the cell AP, which is is recorded as a compensatory current. After measurement, the analysis the compensation current is subtracted from the reference current to obtain our inhibited ion current. To define the sodium currents for the perfusion solution either tetrodotoxin (TTX, 10 μM) or Anemone toxin II (ATX-II, 1, 10 nM) was added to the perfusion solution. To investigate the role of calcium current in the L-type calcium

channel blocker nizoldipine (1 μM) was used. For all inhibitors we waited for the stabilisation of the pharmacological effect (4-5 min), and the current signal was then the last 20 cycles of this time were taken as the average of the compensatory current in the between the measurement products and the cycles and finally subtracted from the reference current.

The onion peeling method

To study the ionic currents of several ions in a cell a version of the APVC technique called the "onion-peeling method" can be used. In this in this case, in fact, the initial perfusion solution of the cells is added cumulatively, with increasing specific inhibitors of a number of different ion channels. However, it is important to the order of the inhibitors used, from the most specific (e.g. E4031) to the least specific agent (e.g. $BaCl_2$), in order to achieve the most accurate results. This is based on our experiments on outward ion currents in cardiac myocytes the order of the measured currents was I_{Kr}, I_{Ks}, I_{to}, I_{K1}. For all farmacons, until stabilisation of the effect the cell was perfused with the respective solution (4-5 min). was taken as the average of the last 20 cycles of the current signals recorded during the perfusion of the current and subtracted from the reference current, which is always the last the compensation current of the substance previously applied (except for the first substance applied), thus obtaining the sensitive current of the inhibitor in question.

Statistics

In the essay Data are expressed as the mean ± SE of the measurement results. A statistical significance of differences was assessed by one-way ANOVA with Tukey hsd test was performed as a post hoc test. This was done by Student's t-test where the difference was considered significant if the p value was less than 0.05.

Results

Changes in late sodium current conductance and current profile in canine, rabbit and guinea pig cardiac myocytes

Native INa.late profiles under control conditions

Dog, rabbit and guinea pig at the beginning of our research we investigated the shape and conductance of the late sodium current in cardiac myocytes. Conductance values associated to $I_{Na,late}$ ($G_{Na,late}$) were calculated by dividing $I_{Na,late}$ values by the driving force for Na^+, defined as a difference of the actual transmembrane potential and Na^+ equilibrium potential, estimated as +85.3 mV ($[Na^+]_o$ = 146 mM, $[Na^+]_i$ = 6 mM, T=310 K). From $I_{Na,late}$ current density was roughly constant during the AP plateau phase but decreased in phase 3, during cell membrane repolarization in dog and rabbit myocytes. In these measurements, the $G_{Na,late}$ steadily decreased throughout. During the plateau phase of AP in guinea pig cells, $I_{Na,late}$ increased steadily, while $G_{Na,late}$ remained unchanged.

In order to compare the individual results $I_{Na,late}$ and $G_{Na,late}$ values were normalized according to their position on the time axis, where APs measured at 90% of the repolarization of the APs (APD_{90}) was considered 100%. Thus, the dog and rabbit $I_{Na,late}$ and even more so GNa,late curves showed a decrescendo shape, i.e. their amplitude was proportional to the AP ascending In contrast, the $I_{Na,late}$ amplitude increased crescendo during the plateau of the AP, and only at the terminal during terminal repolarization, while $G_{Na,late}$ did not showed no change at this stage.

We measured the $I_{Na,late}$ and $G_{Na,late}$ of 20, 50 and 80 percent of the APD_{90} value for dogs, rabbits and guinea pigs. cells. The conductance changes suggest that $G_{Na,late}$ is greater in dog and rabbit cardiac myocytes than in guinea pig cells. The extent of $G_{Na,late}$ decline in the decline of GNa,late was calculated using the APD_{90} 20 to 80 percent of the APD90 and then to the GNa,late measured at 20 percent of the APD_{90} (abbreviated as "decay factor" = (G20%-G80%)/G20%). This decay factor actually turned out to be lower than the guinea pig muscle cells (-0.07±0.16, n=18) than in the dog (0.46±0.06, n=15), or in the rabbit (0.60±0.04 n=6). Although the $G_{Na,late}$ decline was faster in rabbits than in dogs, this difference did not prove to be significant. The amount of charge transported by $I_{Na,late}$ (taken as the integral of the mean current curves) was approximately equal to in rabbit (-66.5±14.6 mC/F) and dog (-64.2±6 mC/F) myocytes, but significantly lower for guinea pig cells (-94.6±6 mC/F).

Effect of the shape of the command signal on the late sodium current

In order to investigate whether the difference between $G_{Na,late}$ profiles is only due to different AP parameters or the result of differences in Na^+ channel gating between species, we used the canonical action potential generated by rabbit and guinea pig cardiomyocytes as a voltage constant for canine cardiomyocytes. Despite the different command APs, the shape of $I_{Na,late}$ continued to show a monotonic decrease characteristic of canine $I_{Na,late}$. Moreover, the integral of the current curves did not change regardless of the different origin of the command APs.

The role of calcium in the regulation of the late sodium current

In order to find out what role $[Ca^{2+}]_i$ in the regulation of $I_{Na,late}$ and whether it has an effect on conductance, we reduced the Ca^{2+} content of the cytosol to 1μM nisoldipine or 10 mM BAPTA. The amplitude of $I_{Na,late}$ was reduced when nizoldipine was used (-330 ± 30 mA/F vs. -457 ± 38 mA/F for at 20% of APD_{90} and -282 ± 38 vs -412 ± 37 mA/F at 50% of APD90 $p < 0.05$, n = 19 vs. n = 15). The effect of BAPTA at all stages of AP significantly altered the amplitude of $I_{Na,late}$ (-277 ± 44 vs. -457 ± 38 mA/F at 20% of APD_{90}, -306 ± 36 vs. -412 ± 37 at 20% of APD_{90} 50% of APD_{90}, and -223 ± 32 vs. -284 ± 34 mA/F at 80% of APD_{90}, $p < 0.05$, n = 11 vs. n = 15). $G_{Na,late}$ was compared to $I_{Na,late}$ amplitude was reduced by nizoldipine (5.0 ± 0.5 mS/F vs. 6.9 ± 0.7 mS/F for APD_{90} at 20% and 4.0 ±0.5 vs. 5.9 ±0.6 mS/F at 50% of APD_{90}), but the change was significant only at 80% of APD_{90} (2.0 ±0.3 mS/F vs. 3.5 ±0.4 mS/F). that nizoldipine is mainly used during the initial phase of AP decreased $G_{Na,late}$, whereas BAPTA decreased $G_{Na,late}$ more in the later parts of the AP. However, current integrals were equally reduced by BAPTA and nizoldipine (-48.5 ±5 mC/F vs. -63.9 ±6 mC/F and -46.7 ±5 vs. -63.9 ±6 mC/F, respectively).

The effect of ATX-II on canine and guinea pig cardiomyocites

ATX-II is the by inhibiting the rapid inactivation of Na^+ channels in an $I_{Na,late}$ - induces a current in the excitable tissues of the heart very similar to that of I_{Na}. On this measurements, we considered the 10 μM TTX-sensitive current to be $I_{Na,late}$. ATX-II was elongated in both canine and guinea pig myocytes and increased the amplitude of $I_{Na,late}$. Whereas $I_{Na,late}$ recorded in the presence and absence of ATX-II were recorded in separate measurements, so only the averaged $I_{Na,late}$ and $G_{Na,late}$ profiles could be compared

without the SEM values. Although the current shape formed by ATX-II did not exactly match the native $I_{Na,late}$, its shape was similar to its native counterpart in both species. In contrast, while in guinea pig cardiac myocytes, the native conductance was roughly constant in the AP plateau of AP, whereas the $G_{Na,late}$ elevation measured with ATX-II trend over the same period. Accordingly, dog $G_{Na,late}$ decay factor in canine cardiomyocytes was dependent on the presence of ATX-II remained similar (0.54 ± 0.06, n = 6 and 0.46 ± 0.06, n = 15). In guineapigs, the decay factor was much lower in the presence of ATX-II, than control (-0.95 ± 0.81, n = 4 vs - .07 ± 0.16, n = 18, $p < 0.05$), i.e., conduction was further increased by the time of AP terminal repolarization.

Beta-adrenergic activation of the inward rectifying potassium current by the CaMKII pathway in left ventricular myocytes of the canine heart:

Variation of I_{K1} parameters during the action potential:

Our series of experiments we investigated the I_{K1} current measured by APVC technique in five groups properties: control, β-adrenergic stimulated with 10 nM ISO, β-adrenergic stimulated but treated with 1 µM KN-93, β-adrenergic stimulated but treated with 3 µM H-89 and β-adrenergic stimulated but treated with both KN-93 and H-89.

β-adrenergic stimulation by ISO significantly increased IK1 current density at half the length of the command AP (IK1 plateau mid density; control: 0.067±0.019 A/F vs ISO: 0.159±0.029 A/F, n=7 for both groups). This effect was not observed in the KN-93 pretreatment inhibited (0.073±0.014 A/F, n=9), but was not affected by H-89 (0.136±0.024 A/F, n=10).When cells were treated with a combination of KN-93 and H-89 pretreatment (0.065±0.013 A/F, n=7), the mean density of the IK1 current plateau approximated those measured in control and KN-93 pretreated cases only. Other parameters of IK1, such as current terminal repolarization peak density during the terminal phase (control: 1.849±0.120 A/F vs ISO: 1.967±0.159 A/F), or the total current integral (control: 66.7±11.7 mC/F vs ISO: 77.6±8.1 mC/F), β-adrenergic stimulation had no significant effect of interest.

To isolate the effect of CaMKII and PKA inhibition on β-adrenergic stimulation, we examined the effect of KN-93 and H-89 on IK1 without ISO. With neither inhibitor did we observe any difference from the control values compared to control values in neither current density nor current integral results.

β-adrenergic effect, focus on the AP plateau

As our previous results suggest that the I_{K1} β-adrenergic signalling pathway during phase 2 of AP, i.e. during the plateau occurs at this section, so we focused the analysis of the results of our previous measurements for this phase. Thus, the current density of I_{K1} is calculated by two fixed membrane potential values of +20 mV, which is the mid-plane membrane potential, and 0 mV, which is measured during the fast phase of repolarization. The current density of I_{K1} is significantly increased by ISO (0.148±0.034 A/F at +20 mV, 0.202±0.041 A/F 0 mV), compared to control values (+20 mV: 0.042±0.011 A/F, 0 mV: 0.090±0.018 A/F). This effect was inhibited by KN-93 (+20 mV: 0.052±0.012 A/F, 0 mV: 0.095±0.028 A/F), but not by H-89 (+20 mV: 0.127±0.025 A/F, 0 mV: 0.159±0.022 A/F).

β-adrenergic stimulated I_{K1} with conventional voltage clamp

From similar results to the previous ones were obtained when ISO and kinase inhibitors effects of kinase inhibitors were investigated using the conventional voltage clamp method at -80 and +20 mV Between -30 and +20 mV the current density of I_{K1} increases significantly β-adrenergic stimulation compared to the control cell group. This change is abolished by inhibition of CaMKII and is unaffected by inhibition of PKA. At 0 mV I_{K1} current density values were as follows: control (n=10): 0.266±0.042 A/F, ISO after pretreatment (n=9): 0.211±0.067 A/F, KN-93 and ISO after pretreatment (n=10): 0.211±0.067 A/F, and H-89 and ISO after pretreatment (n=9): 0.431±0.103 A/F. no membrane potential values more negative than -30 mV were observed significant differences.

I_{Kr} and β-adrenergic stimulation

In our research we have investigated both ISO and the above mentioned kinase inhibitors (KN-93, H-89) on I_{Kr}, a major repolarizing current in ventricular myocardial cells under the same conditions as before (alone and in combination). Neither β-adrenergic stimulation nor its inhibition had any effect on I_{Kr} current profile, current density, and current integral values.

Discussion

Interspecies differences in the properties of the late sodium current

Species dependence of the conductance of the late sodium current

Dog and rabbit muscle cells, conductance monotonically decreased during the AP plateau phase phase. In contrast, the $G_{Na,late}$ of guinea pig cardiac myocytes remained unchanged during the same phase of AP, but showed a sharp decrease terminal repolarization phase. These differences in conductance changes may explain why higher amplitude of the AP of $I_{Na,late}$ in guinea pig myocytes during the plateau phase than in dog or rabbit myocytes. The sodium channels inactivation, we introduced a decay factor to monitor the inactivation of the significantly higher in rabbit and dog cells, indicating that Na^+ channels are already inactivated for terminal repolarization in these species. As a result, the the increased driving force for the terminal repolarization phase cannot really increase the amplitude of $I_{Na,late}$ in dogs and rabbits, but it can in guinea pigs. This is explained by the fact that in guinea pigs the time constant of the $I_{Na,late}$ incativity at 20 mV using the conventional voltage clamp method, is 2.5 times longer than in dogs. The same $G_{Na,late}$ the concentration of Na^+ in the pipette internal solution (6 mM) was taken as the cell's intracellular Na+ concentration of the intracellular Na in the cells we used, but the subsarcolemmal sodium concentration of the cells we used can reach 8-9 mM. Consequently, as the Na^+ concentration increases, its equilibrium potential decreases. The smaller the reversal potential generates less driving force for sodium, which leads to higher Na^+ permeability in the calculations. Since similar approximate Na^+ concentrations were used for all three species, no large differences in the dynamics of the concentrations for these three species, and therefore probably underestimated the $G_{Na,late}$ of all three species by similar amounts. Thus, it can be assumed that the presence of $I_{Na,late}$ is more likely to be due to the Na^+ permeability rather than the actual [Na^+].

The relationship between $I_{Na,late}$ and action potential

The monotonic increase in $I_{Na,late}$ of guinea pig ventricular myocytes during AP plateau can be explained by non-equilibrium gating of sodium channels. According to this model, $I_{Na,late}$ "builds up" during phase 2 of AP due to slow, ramp-like repolarization. Contrary to our expectations, however, the profile of late sodium current did not change in canine myocardial cells, whether AP recorded from guinea pig or rabbit ventricular cells was used as command

potential. In this context, it is also worth bearing in mind that the $I_{Na,late}$ and $G_{Na,late}$ profiles of rabbit and guinea pig myocardial cells differ significantly. For this reason, we conclude that the monotonically increasing current profile observed in guinea-pig muscle cells is due to a combination of increased propulsion during repolarization and the effects of slow Na^+ channel inactivation kinetics. Interspecies differences in the inactivation kinetics of late sodium current are therefore worth further

The aritmogenic effect of $I_{Na,late}$

As the profile of $I_{Na,late}$ shows significant differences between species, it is concluded that the shape of AP also varies as APD changes. The monotonically increasing current of guinea pig cardiac myocytes suggests that its contribution to the action potential increases with the elongation of APD. Since the current density reaches its maximum at the final stage of repolarization, if the APD is prolonged for any reason, for example due to K^+ channel blockers, this will result in an increased inward current $I_{Na,late}$. This can stretch the APD further in a backward direction. In addition, the Na^+ and Ca^{2+} load due to the increased $I_{Na,late}$ may be a substrate for arrhythmias.

In contrast, the $I_{Na,late}$ current density of rabbit and canine myocardial cells, which shows a monotonic decrease towards terminal repolarization, contributes less and less to AP formation during prolonged APD. This is also true the other way round, due to the low terminal current density, the change in APD will not have a significant effect on $I_{Na,late}$ in canine and rabbit ventricular myocytes. Concomitantly, mechanisms that reduce $I_{Na,late}$ also have a minor role in dog or rabbit cardiac myocytes. This should be taken into account in pharmacological and electrophysiological studies using guinea pig cardiac myocytes as a model for $I_{Na,late}$.

Properties of the ionic current generated by ATX-II in guinea pig and dog cardiac myocytes

Our studies on late sodium current also showed that as the current generated by ATX-II increases during the AP plateau phase, the shape of ATX-II-induced $G_{Na,late}$ shows a significant difference compared to native conductance in guinea pig cardiac myocytes assayed by APVC technique. This unexpected difference in conductance could represent the binding of ATX-II to Na^+ channels, in which case the difference should also be present in canine cardiomyocytes. Another explanation is the previously mentioned non-equilibrium gating of sodium channels.

Since ATX-II is known to inhibit the inactivation of Na^+ channels, it is a commonly used tool to simulate pathological increases in $I_{Na,late}$. Because of our previously described results, the use of ATX-II for this purpose may lead to rather misleading results in experiments on guinea pig ventricular myocytes. In addition, ATX-II binding may also alter the drug sensitivity of guinea pig muscle cell Na^+ channels, making it difficult to interpret the results of experiments on guinea pig preparations.

Effect of β-adrenergic stimulation on the potassium currents in ventricular myocardial cells

CaMKII pathway responsible for β-adrenergic effects

In our experiments, we investigated the effect of β-adrenergic stimulation on the two main potassium currents responsible for the terminal repolarization of AP, I_{K1} and I_{Kr}, by perfusing myocardial cells with 10 nM ISO. The amplitude of I_{K1} was increased by ISO treatment, but I_{Kr} current parameters were not changed. Our series of experiments showed for the first time that PKA is not responsible for the effects of the β-adrenergic signaling pathway on IK1 in dogs, as the effect of ISO was not altered by pretreatment with the PKA inhibitor H-89. In contrast, the CaMKII inhibitor KN-93 abolished the effect of ISO, suggesting that β-adrenergic stimulation exerts its current-enhancing effects via the CaMKII enzyme. We can also state that there is no synergy between the two pathways (PKA, and CaMKII), as when the two kinase inhibitors were used in combination, our results were not different from when KN-93 was used alone.

As to how CaMKII enhances I_{K1}, two possibilities emerge from the literature. In a previous study, β-adrenergic stimulation increased CaMKII activity in a NO-dependent but cAMP-independent manner in guinea pig cardiac myocytes. This suggests the presence of a novel CaMKII activation pathway via NO synthase. In addition, in rodent cardiac myocytes, the effect of 100 nM ISO is mediated by nitrosylation of the CaMKII delta, but NO also increases CaMKII activity and $K_{ir}2.1$ current density in human atrial myocytes. Several studies have also shown a role for the EPAC protein in CaMKII activation. In rabbit and rodent cardiac myocytes, it has been suggested that CaMKII is activated via a cAMP -> EPAC -> NO -> CaMKII sequence, but an alternative cAMP-> EPAC -> Rap -> PLC-epsilon sequence has also been discovered. Furthermore, the role of β-arrestins and their associated signalosomes cannot be excluded.

β-adrenergic stimulation also only partially affects I_{K1}

In our study, we found that ISO also increased I_{K1} current density only in the range of membrane potentials corresponding to the AP plateau phase, but at values more negative than that, it did not affect it. This finding suggests that the β-adrenergic effect does not affect either the number of active $K_{ir}2.1$ channels or their permeability. Our results suggest that the inward rectifying property of the channels is reduced by phosphorylation by CaMKII, so that a larger outward I_{K1} current can be generated in the positive membrane potential range. A similar phosphorylation-dependent modification was observed in R67Q mutation of $K_{ir}2.1$/KCNJ2 channels, which reduced the ISO-induced effects on I_{K1}.

The results of a study by Nagy et al. are in agreement with our measurements of calcium-dependent enhancement of I_{K1} by CaMKII. However, there was a significant difference between the two studies: while in our results ISO only increased the amplitude of I_{K1} during the AP plateau, in the previous study the peak current density was also increased. The difference is probably due to the fact that while in our experiments calcium levels were altered by β-adrenergic stimulation alone, Nagy et al. also investigated the calcium dependence of I_{K1} at low and high $[Ca^{2+}]_i$, which was achieved by using BAPTA and $CaCl_2$ applied inside the patch pipette.

Under experimental conditions similar to ours, but in rabbit ventricular myocytes, Hegyi et al. also found that inhibition of CaMKII does not affect I_{K1} parameters and that the application of 10 nM ISO does not change the peak current density. These data are in agreement with our measurements, however, the effect of ISO significantly increased the charge carried by I_{K1} compared to control conditions. This significant change was not observed in our measurements. The authors did not address which part of the I_{K1} current variation explains the significant increase in the current integral, but since the peak density did not change, the increase in I_{K1} amplitude below the plateau may explain this phenomenon, which is also consistent with our description. After buffering of calcium by BAPTA, Hegyi et al. found no significant difference in either I_{K1} peak density or net charge delivered by the current after β-adrenergic stimulation. In our study, blockade of CaMKII by KN-93 led to similar results: KN-93 prevented the increase in I_{K1} plateau amplitude under 10 nM ISO, and the current integral was found to be similar to that measured under control conditions. The observations of both studies can be explained by the fact that β-adrenergic stimulation activates the I_{K1} current by CaMKII. It is widely accepted that I_{K1} is essentially activated by phosphatidylinositol-4,5-bisphosphate (PIP2) and that β-adrenergic stimulation leads to an increase in PIP2 concentration. In the study

by Xu and colleagues, the authors propose that activation of β-adrenergic receptors through PKA-dependent phosphorylation and subsequent activation of phosphatidylinositol-4-phosphate 5-kinase gamma (PIP5Kγ) increases PIP2 levels in cardiac myocytes. It should be noted, however, that the authors used 30 μM H-89 extracellularly to inhibit PKA. H-89 is a potent and selective inhibitor of β1 (and β2) adrenergic receptor ligand binding. This β-receptor inhibitory property should be taken into account when H-89 is applied extracellularly. Thus, the results of Xu et al. can be attributed to β-adrenergic receptor inhibition by H-89. This was also the reason why we applied H-89 in the pipette internal solution, intracellularly, and at a much lower concentration of 3 μM. Taking all this into account, an alternative explanation for our results, besides the hypothesized CaMKII-dependent phosphorylation of K_{ir} channels, is possible. Since β-adrenergic receptor activation increases PIP2 levels, it may also enhance IK1 activity through this mechanism. Since in our experiments intracellularly applied KN-93 prevented I_{K1} upregulation, but H-89 had no such effect, this putative mechanism is clearly not mediated by PKA, and CaMKII may also play a role in it.

β-adrenergic stimulation has no effect on I_{Kr}

Compared to I_{Ks} and I_{K1}, the current density of I_{Kr} was not altered by β-adrenergic activation with ISO. In addition, neither CaMKII nor PKA inhibition had any effect on current. Heath et al. reported that in guinea pig cardiac myocytes, IKr current density was increased by activation of PKC, but Klare et al. found that activation of PKA decreased it. In contrast, activation of the PKA pathway increased I_{Kr} amplitude in dog but decreased it in human and rat cardiac myocytes. Since both the PKA and PKC signaling pathways are dependent on $[Ca^{2+}]_i$, it is not surprising that calcium would have a significant influence on the magnitude of the aforementioned effects. Similarly, when $[Ca^{2+}]_i$ was kept low (using EGTA and nifedipine), ISO treatment increased I_{Kr} current density. Likewise, ISO increased I_{Kr} current density at low $[Ca^{2+}]_i$ (with calcium buffer EGTA and Ca2+ channel inhibitor nifedipine) in conventional voltage clamp experiments, but was unchanged in this case (intact calcium homeostasis, APVC technique). By comparing the previous literature data with the results of our study, we conclude that β-adrenergic stimulation of the canine heart does affect I_{Kr}, but presumably through at least two opposing pathways that cancel each other out under normal calcium homeostasis.

Summary

The regulation of the ion channels, and ionic currents of the left ventricular cardiomyocites is a widely researched topic for a long time. There were a lot of developement in this area, however there are still a lot of unanswered questions. Like most biological systems, the regulation of the ion channels have more levels. On one hand, long term regulation is reached through gene expression, which leads to adaptation to habitat and lifestyle, on the other hand short term regulation can be done through the vegetative nervous system, to help and answer to more acute factors.

The aim of our studies were to research, and compare the late sodium currents of three, frequently used modell animals: dog, rabbit and guinea pig. We did our research in control environment, and we studied the similarities and differences in the calcium dependence of the late sodium current between the species. Beside of this, we researched the way the β-adrenergic stimulation affects the potassium currents of the canine left ventricular myocites, to find out if it works through the CaMKII or the PKA mediated pathway.

We did our research with the action potential voltage clamp technique. With this we compared the INa,late currents of dog, rabbit and guinea pig cardiomyocites, and tried to find out if the contour of the trigger action potential affects the profile of the current. We used this technique to research the background of the β-adrenergic regulation of the potassium currents of the canine cardiomyocites.

The results of our research showed that the INa,late of the rabbit and dog cardiomyocites are largely similar, and gets smaller as the AP progresses, however in guinea pig cells the current kept growing. We found out that the contour of the trigger AP doesn't affect the INa,late, and that the ATXII has a similar effect on canine and guinea pig cardiomyocites to the TTX.

In the other half of our studies, we found out, that the β-adrenergic stimulation mostly increase the IK1 current of canine cardiomyocites, and has this effect thorugh the CaMKII pathway, and not by the PKA mediated one. We found out that he IKr is not changed by the β-adrenergic stimulation.

Even if these results don't answer all of our questions about the regulation of the ionic currents of the cardiomyocites, we got a few steps closer to create the whole picture.

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List of publications related to the dissertation

1. **Kovács, Z. M.**, Horváth, B., Dienes, C., Óvári, J., Kiss, D. Z., Hézső, T., Szentandrássy, N., Magyar, J., Bányász, T., Nánási, P. P.: Beta-Adrenergic Activation of the Inward Rectifier K+ Current Is Mediated by the CaMKII Pathway in Canine Ventricular Cardiomyocytes.
Int. J. Mol. Sci. 25 (21), 1-14, 2024.
DOI: http://dx.doi.org/10.3390/ijms252111609
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2. Horváth, B., **Kovács, Z. M.**, Dienes, C., Óvári, J., Szentandrássy, N., Magyar, J., Bányász, T., Varró, A., Nánási, P. P.: Conductance Changes of Na+ Channels during the Late Na+ Current Flowing under Action Potential Voltage Clamp Conditions in Canine, Rabbit, and Guinea Pig Ventricular Myocytes.
Pharmaceuticals (Basel). 16 (4), 560, 2023.
DOI: http://dx.doi.org/10.3390/ph16040560
IF: 4.3

List of other publications

3. Horváth, B., **Kovács, Z. M.**, Dienes, C., Barta, Z., Óvári, J., Szentandrássy, N., Magyar, J., Bányász, T., Nánási, P. P.: Relationship between ion currents and membrane capacitance in canine ventricular myocytes.
Sci. Rep. 14 (1), 11241, 2024.
DOI: http://dx.doi.org/10.1038/s41598-024-61736-6
IF: 3.8 (2023)

4. **Kovács, Z. M.**, Óvári, J., Dienes, C., Magyar, J., Bányász, T., Nánási, P. P., Horváth, B., Fehér, Á., Varga, Z., Szentandrássy, N.: ABT-333 (Dasabuvir) Increases Action Potential Duration and Provokes Early Afterdepolarizations in Canine Left Ventricular Cells via Inhibition of IKr.
Pharmaceuticals (Basel). 16 (4), 488, 2023.
DOI: http://dx.doi.org/10.3390/ph16040488
IF: 4.3

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5. Naveed, M., Mohammed, A. S. A., Topal, L., **Kovács, Z. M.**, Dienes, C., Óvári, J., Szentandrássy, N., Magyar, J., Bányász, T., Prorok, J., Jost, N., Virág, L., Baczkó, I., Varró, A., Nánási, P. P., Horváth, B.: Selective Inhibition of Cardiac Late Na+ Current Is Based on Fast Offset Kinetics of the Inhibitor.
Biomedicines. 11 (9), 2383, 2023.
DOI: http://dx.doi.org/10.3390/biomedicines11092383
IF: 3.9

6. Dienes, C., **Kovács, Z. M.**, Óvári, J., Szentandrássy, N.: TRPM4-ioncsatornák vizsgálatának farmakológiai lehetőségei = Pharmacological possibilities of testing TRPM4 ion channels.
Cardiol. Hung. 53 (5), 446-450, 2023.
DOI: http://dx.doi.org/10.26430/CHUNGARICA.2023.53.5.446

7. Horváth, B., Szentandrássy, N., Dienes, C., **Kovács, Z. M.**, Nánási, P. P., Chen-Izu, Y., Izu, L. T., Bányász, T.: Exploring the Coordination of Cardiac Ion Channels With Action Potential Clamp Technique.
Front. Physiol. 13, 864002, 2022.
DOI: http://dx.doi.org/10.3389/fphys.2022.864002
IF: 4

8. Horváth, B., Szentandrássy, N., Almássy, J., Dienes, C., **Kovács, Z. M.**, Nánási, P. P., Bányász, T.: Late Sodium Current of the Heart: where Do We Stand and Where Are We Going?
Pharmaceuticals (Basel). 15 (2), 231, 2022.
DOI: http://dx.doi.org/10.3390/ph15020231
IF: 4.6

9. **Kovács, Z. M.**, Dienes, C., Hézső, T., Almássy, J., Magyar, J., Bányász, T., Nánási, P. P., Horváth, B., Szentandrássy, N.: Pharmacological Modulation and (Patho)Physiological Roles of TRPM4 Channel-Part 1: modulation of TRPM4.
Pharmaceuticals (Basel). 15 (1), 81, 2022.
DOI: http://dx.doi.org/10.3390/ph15010081
IF: 4.6

10. Dienes, C., **Kovács, Z. M.**, Hézső, T., Almássy, J., Magyar, J., Bányász, T., Nánási, P. P., Horváth, B., Szentandrássy, N.: Pharmacological Modulation and (Patho)Physiological Roles of TRPM4 Channel-Part 2: TRPM4 in Health and Disease.
Pharmaceuticals (Basel). 15 (1), 40, 2022.
DOI: http://dx.doi.org/10.3390/ph15010040
IF: 4.6

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11. Dienes, C., Hézső, T., Kiss, D. Z., Baranyai, D., **Kovács, Z. M.**, Szabó, L., Magyar, J., Bányász, T., Nánási, P. P., Horváth, B., Gönczi, M., Szentandrássy, N.: Electrophysiological Effects of the Transient Receptor Potential Melastatin 4 Channel Inhibitor (4-Chloro-2-(2-chlorophenoxy)acetamido) Benzoic Acid (CBA) in Canine Left Ventricular Cardiomyocytes.
Int. J. Mol. Sci. 22 (17), 9499, 2021.
DOI: http://dx.doi.org/10.3390/ijms22179499
IF: 6.208

12. Horváth, B., Kiss, D. Z., Dienes, C., Hézső, T., **Kovács, Z. M.**, Szentandrássy, N., Almássy, J., Magyar, J., Bányász, T., Nánási, P. P.: Ion current profiles in canine ventricular myocytes obtained by the "onion peeling" technique.
J. Mol. Cell. Cardiol. 158, 153-162, 2021.
DOI: http://dx.doi.org/10.1016/j.yjmcc.2021.05.011
IF: 5.763

13. Kiss, D. Z., Horváth, B., Hézső, T., Dienes, C., **Kovács, Z. M.**, Topal, L., Szentandrássy, N., Almássy, J., Prorok, J., Virág, L., Bányász, T., Varró, A., Nánási, P. P., Magyar, J.: Late Na+ Current Is [Ca2+]i-Dependent in Canine Ventricular Myocytes.
Pharmaceuticals (Basel). 14 (11), 1142, 2021.
DOI: http://dx.doi.org/10.3390/ph14111142
IF: 5.215

Total IF of journals (all publications): 56,186
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The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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