SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

Effects of β-adrenergic stimulation on delayed rectifier potassium currents in canine ventricular cardiomyocytes

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INTRODUCTION

Delayed rectifier potassium currents are essential in cardiac repolarization. Changes in the structure or pharmacological modification of these channel-proteins lead the alteration to the current, the action potential duration and therefore may evoke arrhythmias (in congenital and acquired long QT syndromes, ventricular tachycardia, torsades de pointes ventricular tachycardia, etc.). Arrhythmias evoked their way, start suddenly, often in early ages and so have an outstanding role in sudden cardiac death. The slow component of delayed rectifier potassium current (I_{Ks}) is known to be enhanced by β-adrenergic stimulation, but data about rapid component of this current (I_{Kr}) are controversial.

In this study we examined the effects of direct β-adrenergic stimulation and the modification of the participant intracellular pathways — PKA, PKC — on the rapid and slow components of delayed rectifier potassium current.

During the experiments isoproterenol was used to activate β-adrenergic receptors and we examined the changes in I_{Kr} amplitude. Using specific receptor blockers we identified the β-adrenergic receptor subtype which is involved in the regulation of the I_{Kr}. The participant intracellular pathways— the PKA, EPAC and PKC pathways were explored by using specific activator or inhibitory molecules.

It is well known that most of the PKC inhibitory molecules have direct ion channel effects, as well. To exclude the possible direct potassium channel blocker effect of PKC inhibitory molecules the experiments were performed on hERG channels expressed by HEK-293 cells, too.

Beside the experiments on I_{Kr} we studied the effects of direct β- adrenergic stimulation on I_{Ks}. I_{Ks} is known to be enhanced by β- adrenergic stimulation therefore our concordant data could be used as self-control.

Summarizing our data and comparing them with our earlier results we could form a complex model on the regulation of the components of delayed rectifier potassium current in canine ventricular cardiomyocytes by β-adrenergic stimulation. However some further questions were obtained from our results: in the future we plan to find the proper explanation for the behavioral differences between I_{Ks} and I_{Kr} during PKA stimulation and also to find the molecular explanation of the direct ion channel effect of PKC inhibitory molecules.

LITERARY REVIEW

Cardiac action potentials are formed by several different ionic currents. These currents are activated during the different phases of action potentials and so have different effects on the morphology of them. Delayed rectifier potassium currents have importance in the process of cardiac action potential repolarization. Pharmacological modification of these currents or structural changes in the channel protein may cause the alteration of the action potential duration and therefore may evoke arrhythmias. The most common types of these arrhythmias are the long QT syndromes. In these syndromes the possibility of early after-depolarization and torsades de pointes ventricular tachycardia is increasing due to the elongation of action potential. It could lead to sudden cardiac death.

Delayed rectifier potassium currents are time and membrane potential dependent. They can be divided into three components by their kinetic parameters, intracellular modulation and drug dependence. These are ultra rapid (I_{Kur}), rapid (I_{Kr}) and slow (I_{Ks}) component.

Ultra rapid component of delayed rectifier potassium current (I_{Kur})

The I_{Kur} is characterized by rapid activation kinetic and shows no or very slow inactivation. In human atrial cardiomyocytes only the 4-aminopyridin sensitive I_{Kur} has been detected but this current has not been identified in ventricular cells, yet.

Slow component of delayed rectifier potassium current (I_{Ks})

I_{Ks} is activated by depolarization and shows no inactivation. The charge carrier of this current is mainly K^+ ion but the ion selectivity of this KCNQ1 channel is not as strong as it is in the case of KCNH2 which is responsible for I_{Kr}. I_{Ks} is known to be enhanced by β-adrenergic stimulation. The mutation of KCNQ1 gene is responsible for the development of 5th type of long-QT syndrome.

Rapid component of delayed rectifier potassium current (I_{Kr})

Ion channels forming I_{Kr} belong to the *erg* subgroup of *the eag* group of voltage dependent potassium channels. Mutations of the KCNH2 genes are responsible for the 2nd and 6th type of long-QT syndrome. I_{Kr} is activated rapidly by depolarization. It was thought for a long time that β-adrenergic stimulation has no effect on I_{Kr} but recently it has been published that cAMP inhibits the current of expressed Kv11.1 channels. Similar results were obtained on guinea pig isolated ventricular myocytes. In contrast to these results some studies presented enhanced I_{Kr} by β-adrenergic stimulation. This effect was explained by the increased intracellular calcium concentration due to PKC activation.

β-adrenergic stimulation

Adrenergic receptors

Adrenergic receptors belong to the 7-transmembrane receptor (7-TM-R) family. All of 7-TM receptors work similarly: agonist binding activates intracellular heterotrimer G-proteins. Adrenergic receptors can be categorized by structure and ligand affinity. We can identify α_1, α_2, β_1, β_2 and β_3-adrenergic receptors.

Intracellular pathways

cAMP/PKA pathway

This pathway is modulated by receptors connected to G_s or G_i proteins. The α subunit of heterotirmer G protein either activates or inhibits adenylate cyclase. Adenylate cyclase increases the intracellular cAMP concentration, the cAMP binds to PKA and activates it. PKA influences numerous cell functions for example transcription, metabolism or regulation of cell cycle and apoptosis. Non activated PKA are composed of four subunits — two catalytic (C) and two regulatory (R) subunits which form homo- or heterodimers. The binding of cAMP to R-subunits causes the disinhibition of catalytic subunits. Two types of PKA are known PKA1 and PKA2. PKA1 can be found mainly dissolved in cytoplasm while PKA2 is anchored to specific cell organelles by AKAP proteins. Anchored PKA exerts its effect only on the molecules next to it.

EPAC pathway

cAMP modulates not only PKA but other cAMP sensitive molecules like EPAC. EPAC is a GEF protein which is able to enhance GTP binding and the

activation of monomer G-proteins. Two types of EPAC are known EPAC1 and EPAC2. Both of them have diverse functions, for example they are involved in R-Ras and Rap1/RhoA activation and in this way EPAC influences the cell migration, cell polarity, cell adhesion and cell shape formation. Through Rap1 EPAC is able to activate PLC and PKC pathway, too.

PLC/PKC pathway

This pathway is activated by receptors connected to G_q proteins. The α subunit of G_q activates PLC which forms IP_3 and DAG from the membrane phospholipids. IP_3 and DAG directly activate PKC and increase intracellular calcium concentration through the stimulation of IP_3-receptors of ER. The released calcium serves as an important messenger in the regulation of many cell functions moreover activating some PKC isoforms.

AIM

The effects of cAMP/PKA or PLC/PKC pathways on I_{Kr} were examined by many research groups. Published data are controversial and species-dependent therefore our aims were the following:

1. To study the effects of β-adrenergic stimulation on the rapid component of delayed rectifier potassium current (I_{Kr}) and its kinetics in canine ventricular cardiomyocytes

2. To identify the proper β-adrenergic receptor subtype

3. To map the intracellular pathways that take part in the regulation of I_{Kr}

4. To study the direct effects of PKC inhibitor molecules (chelerythrine, GF109203X) on hERG channels

5. To study the effects of β-adrenergic stimulation on the slow component of delayed rectifier potassium current (I_{Ks}) and to use data as self-control.

METHODS

Single canine ventricular myocytes were obtained from hearts of adult mongrel dogs using the segment perfusion technique. After the chest had been opened, the heart was rapidly removed and the left anterior descending coronary artery was perfused using a Langendorff apparatus. Ca^{2+}-free JMM solution was used during the initial 5 min of perfusion to remove Ca^{2+} and blood from the tissue. Cell dispersion was performed for 30 min in the same solution containing, in

addition, collagenase, bovine serum, albumin and $CaCl_2$. During the isolation procedure, the solutions were gassed with carbogen and the temperature was maintained at 37°C. The obtained isolated cells were rod shaped and showed clear striation when the external calcium was restored. I_{Kr} and I_{Ks} were recorded at 37°C from Ca^{2+}-tolerant canine ventricular cells superfused with oxygenated Tyrode's solution.

This superfusate was supplemented with 5 mM nifedipine plus 1 mM E-4031 when measuring I_{Ks}, or 5 mM nifedipine plus 1 mM HMR-1556 when recording I_{Kr}. Suction pipettes, fabricated from borosilicate glass, had tip resistances of 1.5–2 MΩ after being filled with pipette solution. Membrane currents were recorded with an Multiclamp 700A amplifier (Axon Instruments Inc., Foster City, CA, USA) using the whole cell configuration of the patch clamp technique. After a high (1–10 GΩ) resistance seal had been established by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by further suction. Ion currents were normalized to cell capacitance. Outputs from the clamp amplifier were digitized using an A/D converter (Digidata-1320, Axon Instruments) under software control (pClamp 9.0, Axon Instruments).

All values presented are arithmetic means±SEM. Statistical significance of differences was evaluated by using one way analysis of variance followed by Student’s t-test for paired or unpaired data, as appropriate. Differences were considered significant when the P value was less than 0.05.

RESULTS

Effect of isoproterenol on I_{Kr}

I_{Kr} was activated by 250 ms depolarizing pulses to +10 mV applied at a rate of 0.05 Hz. I_{Kr} was characterized as tail current amplitudes determined as the difference between the peak current and the pedestal value observed following repolarization to the -40 mV holding potential. Exposure of myocytes to isoproterenol for 3–4 min increased I_{Kr} tail amplitude in a readily reversible manner. These current tails were fully eliminated by 1 mM E-4031; therefore, the current can be considered to be purely I_{Kr}. The isoproterenol-induced enhancement of I_{Kr} was caused by a leftward shift in the voltage-dependence of activation of I_{Kr}. The stimulating effect of isoproterenol on I_{Kr} was concentration dependent; 1 mM isoproterenol increased I_{Kr} by 37±3% ($P<0.05$, $n=5$). By fitting these results to the Hill equation, an EC_{50} value of 13.6±2.5 mM and a Hill coefficient of close to unity were obtained.

Responsible β-adrenergic receptor subtype

The effect of isoproterenol was fully prevented by pretreatment with 300 nM CGP-20712A – is known to be selective inhibitor of β_1-adrenoceptors at this concentration. This result suggests that may β_1-adrenoceptors are responsible for mediating activator effect.

Effect of PKA modulation on I_{Kr}

The signal transduction pathway mediating the isoproterenol induced stimulation of I_{Kr} was investigated using specific PKA activators and inhibitors. The effect of isoproterenol was mimicked (i.e. I_{Kr} was increased in a similar extent) by various types of PKA activators, including 3 mM forskolin, 250 mM intracellular cAMP or 8-Br-cAMP. Similar results were obtained with 100 mM 6-bnz-cAMP (selective PKA activator with no effect on EPAC), while the same concentration of 8-pCPT-2'- O-Me-cAMP, a cAMP analogue known to activate EPAC without altering the activity of PKA, failed to enhance I_{Kr}. Intracellular application of 100 mM Rp-8-BrcAMP, a cAMP analogue that is a selective PKA inhibitor, had no effect on baseline I_{Kr}.

The effect of isoproterenol on I_{Kr} was fully prevented by pretreatment with some PKA activators (forskolin, 8-Br-cAMP and 6-bnz-cAMP), and by the PKA inhibitor (Rp-8-Br-cAMP). It was only partially eliminated in the presence of cAMP, while the EPAC activator 8-pCPT-2'-O-Me-cAMP had no effect. These results indicate that the isoproterenol-induced enhancement of I_{Kr} is critically dependent on the activation of PKA.

Effect of PKC modulation on I_{Kr}

Exposure of myocytes to the PKC inhibitor GF109203X (1 μM) decreased I_{Kr} tail amplitude. Similar results were obtained with another PKC inhibitor chelerythrine (1 μM). Many PKC inhibitors are known as direct channel blockers. To exclude this effect of PKC inhibitors these experiments were performed on hERG channels expressed by HEK-293 cells, too.

Both PKC inhibitors caused a concentration-dependent suppression of the hERG current. Fitting data to the Hill equation, the estimated EC_{50} values were 0.76±0.04 and 0.11±0.01 μM for GF109203X and chelerythrine, respectively, with the corresponding Hill coefficient of 1.21±0.07 and 1.52±0.18. Both GF109203X and chelerythrine modified the gating kinetics of the hERG current. The voltage dependence of activation was shifted towards more negative voltages by the two PKC inhibitors. Half-activation voltage, determined by fitting data to the two-state Boltzmann model, was shifted from 2.7±1.2 to −9.5±1.4 mV by 1 μM GF109203X and from 0.6±2.7 to −12.6±3.7 mV by 0.1 μM chelerythrine ($P<0.05$, n=4 for each drug). Time constant of activation was reduced from 301±57 to 143±22 ms and from 247±19 to 146±5 ms by 1 μM GF109203X and 0.1 μM chelerythrine, respectively ($P<0.05$, n=4 for each drug). GF109203X (1 μM) significantly increased the fast and slow time constants of deactivation, while 0.1 μM chelerythrine left these time constants unaffected.

Direct effect of PKC modulation on I_{Kr}

Although the direct blockade of hERG current by chelerythrine and GF109203X was demonstrated above, the effect of PKC inhibition had to be also examined. In these experiments, a lower (0.1 μM) concentration of GF109203X, blocking PKC effectively with relatively small direct blocking action on I_{Kr}, was used. The effect of 0.1 μM GF109203X was tested using both low cytosolic Ca^{2+} concentration and high cytosolic Ca^{2+} level. This was designed to distinguish between possible effects on the calcium-sensitive conventional and calcium-insensitive novel PKC isoforms. Inhibition of PKC by 0.1 μM GF109203X failed to alter the I_{Kr} tail amplitude significantly, and this effect was independent of the actual level of intracellular Ca^{2+}. Similarly, I_{Kr} was

not modified significantly by activation of PKC by 0.1 μM PMA. These results strongly suggest that PKC inhibition itself may has no effect on the amplitude of I_{Kr} in canine ventricular cells.

Effect of β-adrenergic stimulation on I_{Ks}

I_{Ks} was activated by 3 s long depolarizing pulses to +30 mV delivered at a rate of 0.1 Hz from the holding potential of -40 mV. Tail currents, obtained after repolarization in the presence of 5 mM nifedipine plus 1 mM E-4031, were used to characterize I_{Ks}. Exposure of myocytes to 10 and 100 nM isoproterenol increased I_{Ks} tail amplitude in a largely reversible manner. The current was fully eliminated by 1 mM HMR-1556, indicating that it was purely I_{Ks}.

Baseline I_{Ks} was significantly reduced by selective inhibition of PKA using 100 mM intracellular Rp-8-Br-cAMP. On the other hand, full activation of PKA by loading the pipette with 250 mM of the non-hydrolysable cAMP derivative 8-Br-cAMP, increased I_{Ks}, which was three times higher than its control value. The effect of isoproterenol on I_{Ks} was reduced in the presence of Rp-8-Br-cAMP, and isoproterenol failed to enhance I_{Ks} any more when PKA was fully activated by 8-Br-cAMP.

For the sake of comparison, effects of the two PKC inhibitors were studied also on I_{Ks}. Exposure of myocytes to GF109203X (1 μM) or chelerythrine (1 μM) caused small, statistically not significant, increases in I_{Ks} tail amplitudes. In the presence of 100 nM isoproterenol amplitude of I_{Ks} was increased so PKC inhibitors were failed to prevent its activator effect.

CONCLUSION

In the present study, the effects of the β-adrenoceptor agonist isoproterenol on the two components of the delayed rectifier K^+ current, I_{Kr} and I_{Ks}, were studied and compared in canine myocytes. This is the first time an enhancement of I_{Kr} by isoproterenol has been demonstrated in canine ventricular cells, which may be an important mechanism of defense against the lengthening of action potentials in the case of β-adrenoceptor stimulation. This isoproterenol-induced enhancement of I_{Kr} seems to be mediated by the activation of PKA, because the effect of isoproterenol was eliminated after either inhibition or full activation of PKA. However, in contrast to our results, I_{Kr} was shown to be reduced following the activation of PKA in oocytes expressing hERG channels. The reason for this discrepancy is not clear; it may be due to the lack of other important members of the underlying signal transduction pathway in the oocytes, but it may also reflect interspecies' differences as well.

It must be noted, however, that cAMP – in contrast to 8-Br-cAMP and 6-bnz-cAMP – failed to fully prevent the action of isoproterenol. This may be explained by the proper compartmentalization of the PKA-channel complex, suggesting that the submembrane phosphodiesterase barrier may limit the accessibility of PKA from the intracellular side. Thus cAMP – but not 8-Br-cAMP – might partially be degraded locally by phosphodiesterase. In strong support of the PKA-dependent enhancement of I_{Kr} is the finding that when the current was observed in the presence of cAMP analogues, it was markedly elevated resulting in permanent activation of the enzyme.

Similar to our results, Heath and Terrar found that I_{Kr} was enhanced by 10 mM isoproterenol in guinea pig ventricular cells if the conditions required to activate the conventional PKC isoenzymes were met, that is, that Ca^{2+} current was not blocked, cytosolic Ca^{2+} was not buffered and the cell interior was not dialyzed.

They concluded that this stimulating effect was mediated via the activation of the PKC pathway, involving crosstalk between PKA and PKC. However, the activation of the conventional PKC isoforms with thymelatoxin was shown to decrease I_{Kr} in oocytes. Furthermore, our experimental conditions did not favor the activation of conventional PKC isoenzymes, as the Ca^{2+} current was blocked by 5 mM nifedipine, the cytosolic Ca^{2+} was strongly buffered by 10 mM EGTA and the cell interior was dialyzed.

Parallel with PKA we studied the contingent role of EPAC in regulation of I_{Kr}. In our experiments the activation of EPAC via β-adrenergic receptors had no significance in the regulation of this current.

Similar to I_{Kr}, I_{Ks} was also equally enhanced by exposure to isoproterenol and intracellular application of 8-Br-cAMP; however, marked differences were observed between I_{Kr} and I_{Ks} in response to isoproterenol after the inhibition of PKA. Rp-8-Br-Camp strongly compromised baseline I_{Ks}, but failed to modify baseline I_{Kr} at all. This may indicate a more marked contribution of the cAMP/PKA pathway to the basal activity of I_{Ks} compared with that of I_{Kr}. On the other hand, pretreatment with Rp-8-Br-cAMP fully prevented the effect of isoproterenol on I_{Kr}, but only decreased it on I_{Ks}. Thus, it appears that a moderate suppression of the cAMP/PKA pathway is sufficient to blunt the effect of isoproterenol on I_{Kr}, which may be the consequence of a less effective stimulus transduction targeting the hERG channel. However, it is also possible that different PKA isoenzymes with different sensitivities to inhibitors are involved in mediating the effects of β-adrenoceptor stimulation to I_{Kr} and I_{Ks}. In summary, I_{Kr}, similar to I_{Ks}, is enhanced by isoproterenol in canine ventricular myocytes via the activation of the cAMP/PKA system.

To study the effects of PKC system on the rapid and slow components of the delayed rectifier K^+ current two frequently used PKC inhibitors, chelerythrine and GF109203X, were used. I_{Kr}—but not I_{Ks}—was strongly suppressed by both

agents. Since the inhibitory effects of chelerythrine and GF109203X were also observed in pure hERG channels, expressed without the co-expression of the members of the PKC system in HEK cells, one may conclude that these drugs block I_{Kr} directly, i.e., independently of their PKC-inhibiting potencies. This is further supported by the findings that (1) the effects of chelerythrine and GF109203X developed rapidly and (2) manipulation of the PKC system by PMA and low concentration of GF109203X failed to alter I_{Kr} significantly. Although GF109203X has been previously reported to block hERG current directly, we are first to report a direct inhibition of hERG current and canine I_{Kr} by another PKC inhibitor, chelerythrine. In addition to their blocking action, chelerythrine and GF109203X caused marked changes in gating kinetics of hERG current, including a negative shift in the voltage dependence of the activation, acceleration of activation, and slowing of deactivation. Direct inhibition of hERG channels is not an exceptional side effect of PKC inhibitors. The hERG-inhibitor effect of GF109203X has been previously described by Thomas et al. presenting results very congruent with ours. The EC_{50} value obtained in the HEK cells was 0.76 μM in ours, while 1 μM in their experiments. GF109203X (1 μM) caused a 69.2% inhibition in the native I_{Kr} of guinea pigs and a 69.4% blockade of canine I_{Kr}. An interesting difference between the results of the two studies can be observed in the kinetic properties of I_{Kr} blockade. We found a marked leftward shift of −12.2 mV in the voltage dependence of I_{Kr} activation in the presence of GF109203X, while only a small, statistically not significant change of −2.9 mV was seen by Thomas et al. The reason for this discrepancy is not clear at present; it might partly be caused by an improvement of voltage control due to the reduction of hERG current amplitudes.

Although the general conclusion of this work is that neither chelerythrine nor GF109203X is really suitable for studying the contribution of PKC in regulation of cardiac delayed rectifier K^+ channels, some cautious remarks on this point can

be made. The low concentration (0.1 μM) of GF109203X, which is known to block PKC effectively, failed to decrease I_{Kr} tail amplitudes in intact canine ventricular cells. Furthermore, activation of PKC by PMA was not able to increase I_{Kr}. Our results suggest that the role of PKC in the regulation of I_{Kr} in canine ventricular cardiomyocytes is negligible.

Regarding the role of PKC in controlling I_{Ks}, published observations are quite controversial and show strong interspecies differences. For instance, the activation of PKC was shown to enhance I_{Ks} in native cardiac cells of the guinea and in oocytes expressing human I_{Ks} channel proteins. In contrast, I_{Ks} was suppressed by PKC activation when the oocytes were transfected with murine or rat I_{Ks} channels. Our results are similar to our data of I_{Kr}, they suggest that the PKC-dependent modulation of I_{Ks} in dogs may be negligible.

SUMMARY

Background and purpose: While the slow delayed rectifier potassium current (I_{Ks}) is known to be enhanced by the stimulation of β-adrenoceptors in several mammalian species, phosphorylation-dependent regulation of the rapid delayed rectifier potassium current (I_{Kr}) is controversial. In the absence of relevant human data we decided to study the effects of β-adrenoceptor stimulation and intracellular pathways on delayed rectifier potassium currents in ventricular cardiomyocytes of the dog.

Experimental approach: In our experiments the effects of isoproterenol (ISO), activators and inhibitors of the protein kinase A (PKA) and protein kinase C (PKC) pathways on I_{Kr} and I_{Ks} was studied in canine ventricular myocytes or expressed hERG channels using the whole cell patch-clamp technique at 37 °C.

Results: I_{Kr} was significantly increased (by 30-50%) following superfusion with ISO, forskolin or the intracellular application of PKA activator cAMP analogues (cAMP, 8-Br-cAMP, 6-Bnz-cAMP). Inhibition of PKA by Rp-8-Br-cAMP had no effect on baseline I_{Kr}. The stimulating effect of ISO on I_{Kr} was completely inhibited by selective β1-adrenoceptor antagonists CGP-20712A, by the PKA inhibitor Rp-8-Br-cAMP or by the PKA activator cAMP analogues.

In comparison, I_{Ks} was increased threefold by the superfusion of ISO, and strongly reduced by the PKA inhibitor Rp-8-Br-cAMP. The ISO-induced enhancement of I_{Ks} was decreased by Rp-8-Br-cAMP and completely inhibited by 8-Br-cAMP.

Our results indicate that PKC inhibitor chelerythrine and GF109203X suppressed the I_{Kr} in canine ventricular cells. The effects of PKC activators or inhibitors on expressed hERG channels suggests that PKC regulation does not contribute significantly to the regulation of I_{Kr}.

Conclusion: Our results indicate that the stimulation of β1-adrenoceptors increases I_{Kr}, similar to I_{Ks}, via the activation of PKA in canine ventricular cells. Our results show that PKC has no importance in the regulation of these currents, and that the used PKC inhibitor molecules are not suitable for selective PKC blockade due to their direct ion channel inhibitory effects.

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List of publications related to the dissertation

1. **Harmati, G.**, Bányász, T., Bárándi, L., Szentandrássy, N., Horváth, B., Szabó, G., Szentmiklósi, J.A., Szénási, G., Nánási, P.P., Magyar, J.: Effects of beta-adrenoceptor stimulation on delayed rectifier K(+) currents in canine ventricular cardiomyocytes.. *Br. J. Pharmacol. 162* (4), 890-896, 2011.
   DOI: http://dx.doi.org/10.1111/j.1476-5381.2010.01092.x
   IF:5.204 (2009)

2. **Harmati, G.**, Papp, F., Szentandrássy, N., Bárándi, L., Ruzsnavszky, F., Horváth, B., Bányász, T., Magyar, J., Panyi, G., Krasznai, Z., Nánási, P.P.: Effects of the PKC inhibitors chelerythrine and bisindolylmaleimide I (GF 109203X) on delayed rectifier K(+) currents.
   Naunyn Schmiedebergs Arch. Pharmacol. 383 (2), 141-148, 2010.
   DOI: http://dx.doi.org/10.1007/s00210-010-0584-8
   IF:2.631 (2009)

List of other publications

3. Szentandrássy, N., **Harmati, G.**, Bárándi, L., Simkó, J., Horváth, B., Magyar, J., Bányász, T., Lőrincz, I., Szebeni, A., Kecskeméti, V., Nánási, P.P.: Effects of rosiglitazone on the configuration of action potentials and ion currents in canine ventricular cells.
Br. J. Pharmacol. 163 (3), 499-509, 2011.
DOI: http://dx.doi.org/10.1111/j.1476-5381.2011.01215.x
IF:5.204 (2009)

4. Szentmiklósi, J.A., Cseppentő, Á., **Harmati, G.**, Nánási, P.P.: Novel trends in the treatment of cardiovascular disorders: Site- and event- selective adenosinergic drugs..
Curr. Med. Chem. 18 (8), 1164-87, 2011.
DOI: http://dx.doi.org/10.1007/s00395-009-0082-7
IF:4.708 (2009)

5. Virág, L., Jost, N., Papp, R., Koncz, I., Kristóf, A., Kohajda, Z., **Harmati, G.**, Carbonell-Pascual, B., Ferrero jr, J.M., Papp, G.J., Nánási, P.P., Varró, A.: Analysis of the contribution of Ito to repolarization in canine ventricular myocardium..
Br. J. Pharmacol. Epub ahead of print (2011)
DOI: http://dx.doi.org/10.1111/j.1476-5381.2011.01331.x
IF:5.204 (2009)

6. Bárándi, L., Virág, L., Jost, N., Horváth, Z., Koncz, I., Papp, R., **Harmati, G.**, Horváth, B., Szentandrássy, N., Bányász, T., Magyar, J., Zaza, A., Varró, A., Nánási, P.P.: Reverse rate-dependent changes are determined by baseline action potential duration in mammalian and human ventricular preparations.
Basic Res. Cardiol. 105 (3), 315-323, 2010.
DOI: http://dx.doi.org/10.1007/s00395-009-0082-7
IF:5.973 (2009)

7. Bárándi, L., **Harmati, G.**, Horváth, B., Szentandrássy, N., Magyar, J., Varró, A., Nánási, P.P., Bányász, T.: Drug-induced changes in action potential duration are proportional to action potential duration in rat ventricular myocardium.
Gen. Physiol. Biophys. 29 (3), 309-313, 2010.
DOI: http://dx.doi.org/10.4149/gpb_2010_03_309
IF:0.741 (2009)

8. Simkó, J., Szentandrássy, N., **Harmati, G.**, Bárándi, L., Horváth, B., Magyar, J., Bányász, T., Lőrincz, I., Nánási, P.P.: Effects of ropinirole on action potential characteristics and the underlying ion currents in canine ventricular myocytes.

Naunyn-Schmiedebergs Arch. Pharmacol. 382 (3), 213-220, 2010.
DOI: http://dx.doi.org/10.1007/s00210-010-0538-1
IF:2.631 (2009)

9. Bányász, T., Horváth, B., Virág, L., Bárándi, L., Szentandrássy, N., **Harmati, G.**, Magyar, J., Marangoni, S., Zaza, A., Varró, A., Nánási, P.P.: Reverse rate dependency is an intrinsic property of canine cardiac preparations.
Cardiovasc. Res. 84 (2), 237-244, 2009.
DOI: http://dx.doi.org/10.1093/cvr/cvp213
IF:5.801

The Candidate's publication data submitted to the Publication Database of the University of Debrecen have been validated by Kenezy Life Sciences Library on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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