CONTRACT RESEARCH ACTIVITY (CRO)

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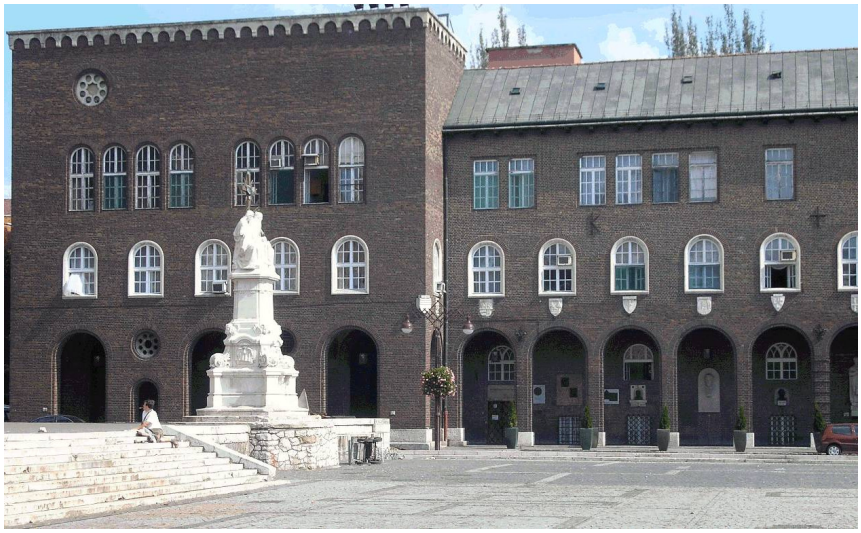

Senior experts:

Profs Julius Gy Papp and András Varró - Cellular Electrophysiology Labs.

Prof Ágnes Végh - In Vivo Haemodynamic Labs.

Prof István Leprán - In Vivo Arrhythmia Labs.

Associate prof János Pataricza - In Vitro Organ Labs.

Associate prof András Tóth – Epifluorescens Ca^{2+} measurement imaging lab.

Associate prof László Virág – Patch-clamp lab.

Assistant prof István Baczkó – In vivo electrophysiological lab.

Biologist Viktória Szűts – Molecular Biology lab.

Expertise on the safety pharmacology of the drug induced long QT syndrome and possible proarrhythmic effects. Studies under GLP conditions.

In vitro methods

a) Voltage clamp caracterization of drug effects on various transmembrane potassium currents in single dog, rabbit and guinea pig cardiomyocytes.

I_{Kr} = rapid delayed rectifier potassium current,

I_{Ks} = slow delayed rectifier potassium current,

I_{k1} = inward rectifier potassium current,

I_{to} = transient outward potassium current,

I_{KATP} = ATP senzitiv potassium current,

I_{NCX} = Na/Ca exchanger current.

This technique can provide important information about the possible effect of new compounds on specific transmembrane currents in freshly isolated native cardiomyocytes.

b) Voltage clamp measurements is HERG, Kv1.5, KvLQT1 + MinK, and Kv 4.2, 4.3, 1.4 expressed mammalian cell lines (CHO, HEK)

References:

Varró A, Takács J, Németh M, Hála O, Virág L, Iost N, Baláti B, Ágoston M, Vereckei A, Pastor G, Delbruyére M, Gautier P, Nisato D and Papp JGy: *Electrophysiological effects of dronedarone (SR 33589), a noniodinated amiodarone derivative in the canine heart: comparison with amiodarone.* Br J Pharmacol 2001;133:625-634.

Virág L, Iost N, Opincariu M, Szolnoky J, Szécsi J, Bogáts G, Szenohradszky P, Varró A and Papp JGy: *The slow component of the delayed rectifier potassium current in undiseased human ventricular myocytes.* Cardiovasc Res 2001;49:790-797.

Varró A, Baláti B, Iost N, Takács J, Virág L, Lathrop DA, Lengyel Cs, Tálosi L. and Papp JGy: *The role of the delayed rectifier component IKs in dog ventricular muscle and Purkinje fiber repolarization.* J Physiol 2000;523.1:67-81.

Virág L, Varró A and Papp JGy: *Effect of disopyramide on potassium currents in rabbit ventricular myocytes.* Naunyn-Schmiedeberg's Arch Pharmacol 1998;357:268-275.

c) Effect of drugs on the action potentials in dog ventricular papillary muscle and cardiac Purkinje fibers examined with the conventional microelectrode technique.

Under GLP – conditions

d) Effect of drugs on the action potentials in dog cardiac subendocardial, subepicardial and midmyocardial cardiac preparations studied by the conventional microelectrode technique.

Reliable technique to detect and screen possible repolarization lengthening or depolarization (sodium current) depressing effects of various substances.

This method can be extended to preparations where the repolarization reserve is attenuated. In these preparations the possible proarrhythmic danger can be detected more accurately.

References:

Takács J, Iost N, Lengyel Cs, Virág L, Nesic M, Varró A, Papp JGy: *Multiple cellular electrophysiological effects of azimilide in canine cardiac preparations.* Eur J Pharmacol 2003;470:163-170.

Varró A, Takács J, Németh M, Hála O, Virág L, Iost N, Baláti B, Ágoston M, Vereckei A, Pastor G, Delbruyére M, Gautier P, Nisato D and Papp JGy: *Electrophysiological effects of dronedarone (SR 33589), a noniodinated amiodarone derivative in the canine heart: comparison with amiodarone.* Br J Pharmacol 2001;133:625-634.

Varró A, Baláti B, Iost N, Takács J, Virág L, Lathrop DA, Lengyel Cs, Tálosi L. and Papp JGy: *The role of the delayed rectifier component IKs in dog ventricular muscle and Purkinje fiber repolarization.* J Physiol 2000;523.1:67-81.

Biliczki P, Virág L, Iost N, Papp JGy and Varró A: *Interaction of different potassium channels in cardiac repolarization in dog ventricular preparations: role of repolarization reserve.* Br J Pharmacol 2002;137:361-368.

Opincariu M, Varró A, Iost NL, Hála O, Szolnoki J, Szécsi J, Bogáts G, Szenohradszky P, Mátyus P and Papp JGy: *The Cellular Electrophysiologic Effect of A New Amiodarone Like Antiarrhythmic Drug GYKI 16638 in Undiseased Human Ventricular Muscle: Comparison With Sotalol and Mexiletine.* Current Medicinal Chemistry 2002;9:41-46.

Baláti B, Varró A and Papp JGy: *Comparison of the cellular electrophysiological characteristics of canine left ventricular epicardium, M cells, endocardium and Purkinje fibres.* Acta Physiol Scand 1998;164:181-190.

e) To establish drug effect on early (EAD) and delayed (DAD) afterdepolarizations in dog cardiac Purkinje and ventricular muscle fibers with the conventional microelectrode technique.

By this method information can be obtained about the possible effect of new compounds on the triggered automaticity at the cellular level.

References

Varró A, Takács J, Németh M, Hála O, Virág L, Iost N, Baláti B, Ágoston M, Vereckei A, Pastor G, Delbruyére M, Gautier P, Nisato D and Papp JGy: Electrophysiological effects of dronedarone *(SR 33589), a noniodinated amiodarone derivative in the canine heart: comparison with amiodarone.* Br J Pharmacol 2001;133:625-634.

Lathrop DA, Nánási PP and Varró A: *In vitro cardiac models of dog Purkinje fibre triggered and spontaneous electrical activity: effects of nicorandil.* Br J Pharmacol 1990;99:119-123.

Varró A and Lathrop DA: *Sotalol and Mexiletine: Combination of Rate-Dependent Electrophysiological Effects.* J Cardiovasc Pharmacol 1990;16:557-567.

f) To investigate and screen the possible QTc lengthening effect of compounds in Langendorf perfused rabbit hearts.′torsadogenic′and

This is a sensitive method to reveal possible QTc lengthening and proarrhythmic effect of compounds selectively inhibiting IKr (HERG + MIRP) current.

References:

Lengyel Cs, Iost N, Virág L, Varró A, Lathrop DA and Papp JGy: *Pharmacological block of the slow component of the outward delayed rectifier current (IKs) fails to lengthen rabbit ventricular muscle QTc and action potential duration.* Br J Pharmacol 2001;132:101-110.

g) To establish the possible 'torsadogenic' effect of a compound in anaesthetized rabbit and dogs in comparison with D-sotalol or dofetilide.

References:

Farkas A, Leprán I and Papp JGy: *Comparison of the antiarrhythmic and proarrhythmic effect of almokakant in anaesthetised rabbits.* European J Pharmacol 1998;346:245-253.

Farkas A, Leprán I and Papp JGy: *The proarrhythmic effects of intravenous quinidine, amiodarone, d-sotalol and almokalant in the anaesthetised rabbit model of torsade de pointes.* J Cardiovasc Pharmacol 2002;39:287-297.

Studies of the possible antiarrhythmic effect of compounds

***a) In vitro* cellular electrophysiological techniques mentioned earlier (under item 1).**

b) Coronary ligation induced arrhythmia model in conscious rats

References:

Leprán I, Koltai M, Siegmund W and Szekeres L: *Coronary artery ligation, early arrhythmias, and determinatuons of the ischemic area in concious rats.* J Pharmacol Meth 1983;9:219-230.

Leprán I, Baczkó I, Varró A and Papp JGy: *ATP--sensitive potassium channel modulators: Both pinacidil and glibenclamide produce antiarrhythmic activity during acute myocardial infarction in conscious rats.* J Pharmacol Exp Ther 1996;277:1215-1220.

Leprán I and Papp JGy: *Effect of long-term oral pretreatment with levosimendan on cardiac arrhythmias during coronary artery occlusion in couscious rats.* Eur J Pharmacol 2003; 464:171-176.

c) Coronary ligation and reperfusion induced arrhythmia models in anaesthetized rats and rabbits

References:

Leprán I and Papp JGy: *Effect of moxonidine on arrhythmias induced by coronary artery occlusion and reperfusion.* J Cardiovasc 1994;24(Suppl.I):S9-S15.

Baczkó I, Leprán I and Papp JGy: *Influence of anaesthetics on sudden death and the incidence and duration of reperfusion-induced arrhythmias in rats.* J Cardiovasc Pharmacol 1997; 29:196-201.

Baczkó I, El-Reyani NE, Farkas A, Virág L, Iost N, Leprán I, Mátyus P, Varró A and Papp JGy: *Abtiarrhythmic and electrophysiological effects of GYKI-16638, a novel N-(phenoxyalkyl)-N-phenylylkylamine, in rabbits.* European J Pharmacol 2000;4004:181-190.

d) Coronaria ligation and reperfusion induced arrhythmias in anaesthetized dogs.

Evaluation of the antiarrhythmic effect in a model of coronary artery occlusion and reperfusion in anaesthetised dogs

This established model provides excellent possibility to test the antiarrhythmic effect of compounds on severe ventricular arrhythmias resulting from acute coronary artery occlusion and reperfusion.

Measured parameters:
Basic haemodynamic parameters (ABP, HR, LVSP, LVEDP, LV dP/dt)

Electrophysiologic parameters: nhomogeneity of electrical activation
eicardial ST-segment changes

Incidence of ventricular arrhythmias (ventricular premature beats [VPBs], ventricular tachycardia [VT; episodes and incidence], ventricular fibrillation [VF] and survival)

References:

Babai L, Szigeti Zs, Parratt JR, and Végh Á: *Delayed cardioprotective effects of excercise in dogs are aminoguanidine sensitive: possible involvement of nitric oxide;* Clinical Science 2002; 102:435-445.

Kis A, Végh Á, Papp JGy and Parratt JR: *Pacing- induced delayed protection against arrhythmias is attenuated by aminoguanidine, an inhibitor of nitric oxide synthase.* Br J Pharmacol 1999;127:1545-1550.

Kaszala K, Végh Á, Papp JGy and Parratt JR: *Time Course of the Protection Against Ischaemia and Reperfusion-induced Ventricular Arrhythmias Resulting from Brief Periods of Cardiac Pacing.* J Mol Cell Cardiol 1996;28:2085-2095.

Végh Á, Komori S, Szekeres L. and Parratt JR: *Antiarrhythmic effects of preconditioning in anaesthezised dogs and rats.* Cardiovas Res 1992;26:487-495.

e)Atrial fibrillation threshold measurements in anaesthetized cats.

The method is useful to detect possible protective effect in experimental atrial fibrillation.

References:

Szekeres L and Papp JGY: *Experimental Cardiac Arrhythmias and Antiarrhythmic Drugs.* Akadémiai Kiadó Budapest,1971.

General pharmacological and other safety pharmacological investigations

a) asic haemodynamic measurements for safety analysis of various compounds

Monitoring of the arterial blood pressure [ABP], heart rate [HR], left ventricular (LV systolic [S], end-diastolic [ED]) pressure [P] and contractile (LVdP/dt) parameters in anaesthetised closed-chest dogs. This model enables fast testing of the cardiovascular effects of compounds.

b) Assessement of the haemodynamic effects of a newly developed cardiovascular compound

This model provides full haemodynamic analysis of a compound developed for cardiovascular application. The experiments are performed in anaesthetised open-chest dogs. There is opportunity to test dynamic function (see *specific parameters*) of the heart using sonomicrometry. This is particularly advised at the assessement of the effects of cardiotonic drugs.

General parameters:	ABP, HR, LVSP, LVEDP, LV dP/dt, aortic flow [AF; for the assessement of cardiac output [CO].
Specific parameters:	LV systolic and diastolic shortening by sonomicrometry. Coronary blood flow measurements with both Doppler and electromagnetic flow probes.

c) Haemodynamic and ECG analysis of drugs in conscious, instrumented dogs

This model provides an opportunity to evaluate drug effects on the various haemodynamic parameters following intravenous and oral administration in conscious dogs.

Measured parameters: ABP, HR, LVSP, LVEDP, LV dP/dt, CO, basic ECG parameters.

Reference:

Udvary É, Papp JGy and Végh Á: *Cardiovascular effects of the calcium sensitizer, levosimendan, in heart failure induced by rapid pacing in the presence of aortic constriction.* Br J Pharmacol 1995;114:656-661.

d) Two coronary artery occlusion-induced severe myocardial ischaemia model

In this model severe myocardial ischaemia is induced by the occlusion of the two main branches of the left coronary artery. This model can be used for the assessement of the effectiveness of antianginal drugs and also for evaluating the cardio-vascular effects of compounds under conditions of ischaemia-induced acute heart failure.

Measured parameters:
Basic haemodynamic parameters (ABP, HR, LVSP, LVEDP, LV dP/dt, aortic flow [AF; for the assessement of cardiac output [CO])
Specific dynamic parameters (LV systolic and diastolic shortening, local segmental shortening)
Coronary blood flow
Epi and endocardial ST-segment alterations

References:

Végh Á, Szekeres L and Udvary É: *Effect of the blood supply to the normal noninfarcted myocardium on the incidence and severity of early postoccluson arrhythmias in dogs.* Basic Res Cardiol 1987;82:159-171.

Végh Á, Komori S, Szekeres L. and Parratt JR: *Antiarrhythmic effects of preconditioning in anaesthezised dogs and rats.* Cardiovas Res 1992;26:487-495.

e) Hemodynamic analyzis (cardiac output, blood and ventricular pressure, heart rate ect.) of compounds in anaesthetized dogs.

References:

Végh Á, Papp JGy, Udvary É. and Kaszala K: *Hemodymanic Effects of Calcium-Sensitizing Agents.* J Cardiovasc Pharmacol 1995; 26 (Suppl 1): S20-S31.

Végh Á and Papp JGy: *Haemodynamic and other effects of sulphonylurea drugs on the heart.* Diabetes Research and Clinical Practice 31 Suppl 1996; S1-S11.

Végh Á, Szekeres L and Udvary É: *Effect of the blood supply to the normal noninfarcted myocardium on the incidence and severity of early postoccluson arrhythmias in dogs.* Basic Res Cardiol 1987;82:159-171.

f) To screen the possible positive and negative inotropic effect of novel substances and to establish the mechanism of action of cardiotonic drugs in isolated guinea pig, rabbit and dog right ventricular papillary muscle.

References:

Virág L, Hála O, Marton A, Varró A and Papp JGy: *Cardiac Electrophysiological Effects of Levosimendan, a New Calcium Sensitizer.* Gen Pharmac 1996;Vol. 27. No.3, pp. 551-556.

g) Effect of compounds on endothelium dependent and independent changes of the vascular tone by isometric tension measurements in isolated arterial, venous and coronary preparations obtained from dogs and pigs.

References:

Krassói I, Paraticza J, Torday L, Kun A and Papp JGy: *Improvement by Phosphoramidon of Damaged Endothelial Function in Porcine Coronary Artery.* Ann Thorac Surg 2000;70:878-82.

Pataricza J, Hőhn J, Petri A, Balogh Á and Papp JGy: *Comparison of the Vasorelaxing Effect of Cromakalim and the New Inodilator, Levosimendan, in Human Isolated Portal Vein.* J Pharmacy & Pharmacol 2000;52:213-217.

Krassói I, Pataricza J, Papp JGy: *Thiorphan enhances bradykinin-induced vascular relaxation in hypoxic porcine coronary artery.* J Pharmacy & Pharmacol 2003; 55: 339-345.

h) Measurement of drug induced nitric oxid release in aqueous solution.

References:

Pataricza J, Penke B, Balogh GE and Papp JGy: *Polarographic Detection of Nitric Oxide Released from Cardiovascular Compounds in Aqueous Solutions.* J Pharmacol and Toxicol Meth 1998;39:91-95.

Detailed description of the cellular electrophysiological methods

Conventional microelectrode technique to transmembrane action potentials (with GLP standard)

Adult mongrel dogs or rabbits of either sex are used. Following anaesthesia (sodium pentobarbital, 30 $mg \cdot kg^{-1}$ administered intravenously), the heart of each animal is rapidly removed through right lateral thoracotomy. The hearts are immediately rinsed in oxygenated modified Locke`s solution containing (in mM): NaCl, 120; KCl, 4; $CaCl_2$, 1.0; $MgCl_2$, 1; $NaHCO_3$, 22 and glucose, 11. The pH of this solution was 7.35 to 7.40 when saturated with 95% O2 and 5% CO2 at 37 ^oC. Left atrial trabeculea, right ventricular papillary muscle or Purkinje strands obtained from either ventricle were mounted individually in a tissue chamber (volume ~40 ml). Each ventricular preparation is initially stimulated at a basic cycle length of 1000 ms (frequency = 1 Hz), using rectangular constant current pulses 2 ms in duration. These stimuli are isolated from ground and delivered through a bipolar platinum electrode in contact with the preparation. At least 1 h is allowed for each preparation to equilibrate after mounting before experimental measurements are initiated. Temperature of the superfusate is kept constant at 37^oC. Transmembrane potentials are recorded using conventional microelectrode techniques. Microelectrodes filled with 3M KCl and having tip resistances of 5-20 MΩ are connected to the input of a high impedance electrometer. (Experimetria intracellular amplifier)

The maximum diastolic potential, action potential amplitude, action potential duration at 50 and 90% of repolarization (APD_{50} and APD_{90}) and the first derivative of transmembrane potential (V_{max}) are automatically measured using a software developed in our laboratory running on a 386-microprocessor based, IBM compatible computer containing an ADA 3300 analogue-to-digital data acquisition board (Real Time Devices Inc., State Collage, PA, U.S.A.) with a maximum sampling frequency of 40 kHz. After control measurements the preparations are superfused for 40 minutes with saline containing the compound under study, and then the electrophysiological measurements are resumed. Attempts are made to maintain the same impalement throughout each experiment. If, however, an impalement become dislodged, adjustment is attempted, and if the action potential characteristics of the re-established impalement deviate by less than 5% from the previous measurement, the experiment continues.

Whole cell configuration of the patch-clamp technique to measure transmembrane ionic currents

One drop of cell suspension is placed within a transparent recording chamber mounted on the stage of an inverted microscope (TMS, Nikon, Tokyo, Japan), and individual myocytes are allowed to settle and adhere to the chamber bottom for at least 10 minutes before superfusion is initiated. HEPES buffered Tyrode's solution serves as the normal superfusate. This solution contains (mM): NaCl 144, NaH_2PO_4 0.33, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 0.53, Glucose 5.5, and HEPES 5.0 at pH of 7.4.

Patch-clamp micropipettes are fabricated from borosilicate glass capillaries (Clark, Reading, UK) using a P-97 Flaming/Brown micropipette puller (Sutter Co, Novato, CA, USA). These electrodes have resistances between 1.5 and 2.5 MΩ when filled with pipette solution containing (in mM): K-aspartate 100, KCl 45, ATP 3, $MgCl_2$ 1, EGTA 10 and HEPES 5. The pH of this solution is adjusted to 7.2 by KOH. Membrane currents are recorded with Axopatch-1D and

200B patch-clamp amplifiers (Axon Instruments, Union City, CA, USA) using the whole-cell configuration of the patch-clamp technique. After establishing a high (1-10 GΩ) resistance seal by gentle suction, the cell membrane beneath the tip of the electrode is disrupted by suction or by application of 1.5 V electrical pulses for 1 - 5 ms. Membrane currents are digitized using a 333 kHz analog-to-digital converter (Digidata 1200, Axon Instruments) under software control (pClamp 8, Axon Instruments). Analyses are performed using pClamp 8 software (Axon).

These experiments would be performed in either cell lines stably transfected with different ion channel genes (HERG, Kv4.2, Kv4.3 Kv1.4, Kv1.5, etc.) or in native cells isolated from dog or rabbit hearts using an enzymatic dissociation procedure (see below). Measuring native ionic currents in native cells, separation of the different currents is needed. Recording K^+ currents in native cells, nisoldipine (1 μM), an L-type Ca^{2+} current (I_{Ca}) blocker, is added to the external solution to eliminate I_{Ca}. The rapid component of the delayed rectifier potassium current (I_{Kr}) is separated from I_{Ks} by using the selective I_{Ks} blocker L-735,821. In case of L-type Ca^{2+} current recording cesium is substituted for potassium in the pipette solution and 3 mM 4-aminopyridine, a K^+ channel blocker, is added to the external solution in order to block potassium currents.

Isolation of left ventricular myocytes from hearts of dog or rabbit

Ventricular myocytes were enzymatically dissociated from hearts of mongrel dogs or rabbits of either sex following anaesthesia (sodium pentobarbital, 30 mg/kg *i.v.*). The hearts were immediately placed in cold (4 °C) normal Tyrode's solution. A portion of the left ventricular wall (containing an arterial branch large enough to cannulate – in case of dogs) or the whole heart through the aorta (in case of rabbits) was then perfused in a modified Langendorff apparatus at a pressure of 60 cm H_2O with solutions in the following sequence: 1) normal Tyrode's solutions (10 min), 2) Ca^{2+}-free solution (composition in mM: NaCl 135, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, HEPES 10, $NaHCO_3$ 4.4, Glucose 10, and taurine 20; pH 7.2 adjusted with NaOH) (10 min), and 3) Ca^{2+}-free solution containing collagenase (type I, 0.66 mg/ml, Sigma Chemical, St. Louis, MO, USA) and bovine serum albumin (fraction V, fatty acid free, 2 mg/ml, Sigma Chemical) (15 min). Protease (type XIV, 0.12 mg/ml, Sigma Chemical) was added to the final perfusate while and another 15 - 30 min of digestion was allowed. Portions of the left ventricular wall judged to be well digested were diced into small pieces and placed in Ca^{2+}-free solution supplemented with $CaCl_2$ (1.25 mM) for 15 minutes. Next, these tissue samples were gently agitated in a small beaker to dislodge single myocytes from the extracellular matrix. During the entire isolation procedure, solutions were gassed with 100% O_2 while their temperatures were maintained at 37 °C. Myocytes were allowed to settle to the bottom of the beaker for 10 minutes, and then the supernatant was replaced with fresh solution. This procedure was repeated three times. Myocytes were maintained at 12 - 14 °C in Tyrode's solution prior to experimentation.

Contract research collaborations and history

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