Antiarrhythmic and Proarrhythmic Potential of Pharmacological Agents

PhD Thesis

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LIST OF PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

I. **Tamás Hézső*, Muhammad Naveed*,** Csaba Dienes, Dénes Kiss, János Prorok, Tamás Árpádffy-Lovas, Richárd Varga, Erika Fujii, Tanju Mercan, Leila Topal, Kornél Kistamás, Norbert Szentandrássy, János Almássy, Norbert Jost, János Magyar, Tamás Bányász, István Baczkó, András Varró, Péter P. Nánási, László Virág, Balázs Horváth; *Mexiletine-like cellular electrophysiological effects of GS967 in canine ventricular myocardium.* Scientific Reports; doi: 10.1038/s41598-021-88903-3. Published 5 May 2021. (*Impact factor 4.9 (D1)*)

II. **Leila Topal*, Muhammad Naveed*,** Péter Orvos, Bence Pászti1, János Prorok, Ákos Bajtel, Tivadar Kiss, Boglárka Csupor-Löfer, Dezső Csupor, István Baczkó, András Varró, László Virág, Norbert Jost; *The electrophysiological effects of cannabidiol on action potentials and transmembrane_potassium currents in rabbit and dog cardiac ventricular preparations.* Archives of Toxicology; https://doi.org/10.1007/s00204-021-03086-0. Published 24 May 2021. (*Impact factor 6.1 (Q1)*)

III. Zsófia Kohajda, László Virág, Tibor Hornyik, Zoltán Husti, Anita Sztojkov-Ivanov, Norbert Nagy, András Horváth, Richárd Varga, János Prorok, Jozefina Szlovák, Noémi Tóth, Péter Gazdag, Leila Topal, **Muhammad Naveed,** Tamás Árpádffy-Lovas, Bence Pászti, Tibor Magyar, István Koncz, Szilvia Déri, Vivien Demeter-Haludka, Zoltán Aigner, Balázs Ördög, Márta Patfalusi, László Tálosi, László Tiszlavicz, Imre Földesi, Norbert Jost, István Baczkó, András Varró. *In vivo and cellular antiarrhythmic and cardiac electrophysiological effects of desethylamiodarone in dog cardiac preparations.* British Journal of Pharmacology. First published: 01 February 2022. https://doi.org/10.1111/bph.15812. (*Impact factor 9.4 (D1)*)

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LIST OF OTHER PUBLICATIONS DURING PhD STUDY

Full Papers:

I. A. Polyák, L. Topal, J. Prorok, N. Tóth, Zs. Kohajda, Sz. Déri, V. Demeter-Haludka, P. Hegyi, V. Venglovecz, A. Sarusi, G. Ágoston, Z. Husti, N. Zombori-Tóth, P. Gazdag, J. Szlovák, T. Árpádffy-Lovas, **M. Naveed,** N. Jost, L. Virág, N. Nagy, I. Baczkó, A. S. Farkas, A. Varró. *Cardiac electrophysiological remodeling associated with enhanced arrhythmia susceptibility in a canine model of elite exercise. eLife. 2023 Feb 23;12:e80710. doi: 10.7554/eLife.80710* (*Impact factor 8.7 (D1)*)

II. Tamás Árpádffy-Lovas, Aiman Saleh A. Mohammed, **Muhammad Naveed,** István Koncz, Beáta Baláti, Miklós Bitay, Norbert Jost, Norbert Nagy, István Baczkó, László Virág, and András Varró. *Species-dependent differences in the inhibition of various potassium currents and*

in their effects on repolarization in cardiac ventricular muscle. Canadian Journal of Physiology and Pharmacology. Published 20 April 2022. DOI: 10.1139/cjpp-2022-0028. (*Impact factor 2.2 (Q2)*)

III. Bence József Pászti, Janos Prorok, Tibor Magyar, Tamás Árpádffy-Lovas, Balázs Györe, Leila Topál, Péter Gazdag, Jozefina Szlovák, **Muhammad Naveed,** Norbert Jost, Norbert Nagy, András Varró, László Virág, Istvan Koncz. *Cardiac electrophysiological effects of ibuprofen in dog and rabbit ventricular preparations: Possible implication to enhanced proarrhythmic risk*. Canadian Journal of Physiology and Pharmacology.16 September 2020**.** Doi.org/10.1139/cjpp-2020-0386. (Published). (*Impact factor 2.2 (Q2)*)

IV. Aiman Saleh A. Mohammed, **Muhammad Naveed,** Norbert Jost. Polysaccharides; *Classification, Chemical Properties, and Future Perspective Applications in Fields of Pharmacology and Biological Medicine (A Review of Current Applications and Upcoming Potentialities.* Journal of Polymers and the Environment; https://doi.org/10.1007/s10924-021-02052-2. Published 27 January 2021. (*Impact factor 4.7 (Q1)*)

V. Gábor Katona, Fakhara Sabir, Bence Sipos, **Muhammad Naveed,** Zsuzsanna Schelz, István Zupkó, Ildikó Csóka. *Development of Lomustine and n-Propyl Gallate Co-Encapsulated Liposomes for Targeting Glioblastoma Multiforme via Intranasal Administration.* Published: 12 March 2022. https://doi.org/10.3390/pharmaceutics14030631. (*Impact factor 6.5 (Q1)*).

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Quotable Abstracts and Presentations:

I. **Muhammad Naveed,** Leila Topal, Janos Prorok, Bence Paszti, Dezso Csupor, Istvan Baczko, Laszlo Virag, Norbert Jost, Andras Varro. *Assessment of proarrhythmogenic risk for cannabidiol using dog and rabbit cardiac preparations: the electrophysiological effects on action potential and transmembrane potassium currents.* Cardiovascular Research, Volume 118, Issue Supplement_1, June 2022, cvac066.021, https://doi.org/10.1093/cvr/cvac066.021. *(Published Conference Abstract)*

II. **Muhammad Naveed,** Leila Topal, Janos Prorok, Bence Paszti, Dezso Csupor, Istvan Baczko, Laszlo Virag, Norbert Jost, Andras Varro. *The electrophysiological effects on action potential, hERG, and transmembrane ionic currents using dog and rabbit cardiac preparation.* Gordon Research Conference on Cardiac Arrhythmia Mechanisms 26/02/2023-03/03/2023 at Grand Galvez in Galveston, Texas, USA.

III. **Muhammad Naveed,** Leila Topal, Janos Prorok, Bence Paszti, Dezso Csupor, Istvan Baczko, Laszlo Virag, Norbert Jost, Andras Varro. Assessment of proarrhythmogenic risk for cannabidiol using dog and rabbit cardiac preparations: the electrophysiological effects on action potential and transmembrane potassium currents. 46th EWGCCE Meeting Saturday, 04 June 15:25 - Monday, 06 June 2022 13:30 CEST (3 Days), Toledo – Spain.

IV. **Muhammad Naveed,** Leila Topal, Janos Prorok, Bence Paszti, Deszo Csupor, Istvan Baczko, Laszlo Virag, Norbert Jost, Andras Varro. The Electrophysiological Effects of Cannabidiol on Action Potential and Transmembrane Potassium Currents in Dog and Rabbit Cardiac Preparations. 14th Conference of New Frontiers in Basic Cardiovascular Research, 25-27 MAY, 2022. Bratislava, Slovak Republic.

V. **Muhammad Naveed,** Leila Topal, János Prorok, Bence Pászti, Dezső Csupor, István Baczkó, László Virág, Norbert Jost, András Varró. *The Electrophysiological Effects of Cannabidiol on Action Potential and Transmembrane Potassium Currents in Dog and Rabbit Cardiac Preparations.* 7th MEETING OF THE EUROPEAN SECTION AND 8th MEETING OF THE NORTH AMERICAN SECTION OF THE INTERNATIONAL ACADEMY OF CARDIOVASCULAR SCIENCES (IACS) Banja Luka, Bosnia, 20-23 September 2021.

VI. **Muhammad Naveed,** Leila Topal, János Prorok, Bence Pászti, Dezső Csupor, Istvan Baczko, László Virág, Norbert Jost, András Varró. *A cannabidiol elektrofi zológiai hatásainak feltérképezése kutya és nyúl akciós potenciálok és transzmembrán ionáramok vizsgálatával.* Conference: A Magyar Kardiológusok Társaságának tudományos folyóirata. October 2021. DOI: 10.13140/RG.2.2.19113.52329. Balatonfured, Hungary.

INTRODUCTION

The heart is one of the vital organs of the body which act as a mechanical pump to supply the blood to other organs. The cardiac contractions, generated by the action potential (a bioelectrical signal), is the key function of the heart and occurred via excitation-contraction coupling process. The action potential (AP) originates and then propagates from the sinus node cells to the whole heart by the active electrophysiological process known as impulse conduction and can be recorded as a difference in the electrical potential between the extra-, and intracellular space. Cardiac AP is an electrical signal which defines the rate & rhythm of the heartbeat, controls cardiac contraction, and propagates the response of heart to changes in internal and external circumstances. The electrical impulse is produced in the sinoatrial node (SAN) and conducted to the atrioventricular (AV) node (AVN) via internodal, where it is delayed. Next, the impulse is propagated via the common bundle (or His bundle), positioned on the top of the ventricular septum, and the right and left bundle branches, respectively situated in both sides of the ventricular septum each, to the Purkinje fibers network which causes the contraction of the ventricular myocardium.

Ventricular repolarization is controlled by a subtle balance between inward and outward currents including I_{Na}, I_{Ca} I_{to}, I_{Kr}, I_{Ks} and I_{K1} currents, respectively. Under usual conditions, attenuation or inhibition of one type of outward K^+ currents cannot be supposed to induce a marked and potentially harmful APD lengthening, since other K^+ currents might have backed up an adequate repolarizing capacity, that may be considered as a “repolarization reserve”. However, in circumstances where the density of one or more types of K^+ channels is diminished by inheritance or remodelling, further suppression of other K^+ channels may cause an unexpected increase in APD prolongation, leading to proarrhythmic reactions.

Disturbances of AP generation, repolarization abnormalities, and/or impairment of impulse conduction may cause changes in the regularity of the heart rhythm called arrhythmias. Broadly, arrhythmia refers to any irregularities in cardiac beating, or to abnormal heart rates, or both. Intrinsically, cardiac arrhythmia combines numerous types of electrical disturbances, ranging from clinically harmless single extrasystolic beats to harmful, life-threatening tachyarrhythmias such as torsade de pointes (TdP) and ventricular fibrillation (VF). Typically, the mechanism of tachyarrhythmia depends on the dynamic interaction of

a provoking trigger, the vulnerable tissue substrate, and the modulating factors, for instance, autonomic balance, plasma electrolytes, and pharmacological treatments, which effect both. The large number of cardiac arrhythmias is due to an enhanced proarrhythmic substrate combined with a trigger. Re-entry is a common mechanism of arrhythmia and does not necessitate abnormal cellular electrophysiology**.** Re-entry is the circulation of electrical impulse inside a closed pathway (the re-entrant circuit), that permits the impulse to constantly stimulate in the site of its origin. This kind of abnormal excitation depends upon numerous conditions: the existence of a unidirectional block inside a conducting pathway, critical timing and the length of the effective refractory period (ERP) of the normal tissue.

Antiarrhythmics are the medications used to overcome abnormally fast rhythms (tachycardias), such as AF, ventricular tachycardia (VT), and supraventricular tachycardia. However, the narrow therapeutic index of antiarrhythmic drugs causes their use clinically challenging. A better understanding of the arrhythmias' mechanisms is just a preliminary step in their appropriate selection. Clinical factors, proarrhythmic risks and side-effect profiles are more meaningful than the cellular mechanisms of actions in drug selection and monitoring. The concept of proarrhythmia is not new and can be described as the escalation of a persisting arrhythmia or the progression of a new arrhythmia resultant to antiarrhythmic drug. Not only diseases, remodelling, disturbances in serum electrolyte, and genetic disorders can contribute to arrhythmogenesis, but so can drugs, i.e., they might exhibit proarrhythmic effects. Drug-induced arrhythmia can be linked to either exorbitant conduction slowing (e.g.; with class Ia and/or Ic Na^+ channel inhibitors) or marked elongation of ventricular APD (e.g.; with class III agents), or both. Ample number of drugs suppress I_{Na} and impulse conduction including class I antiarrhythmic drugs like quinidine, flecainide etc. These drugs, regardless of the fact that used to treat arrhythmias, may also provoke ventricular tachyarrhythmia, apparently by adapting areas in impaired tissue into areas with unidirectional impulse conduction block (one of the prerequisite of re-entry arrhythmias, i.e., developing the substrate for arrhythmogenesis). The other proarrhythmic drug effect is ventricular repolarization's elongation. This proarrhythmic mechanism can develop both the arrhythmic triggers (by inducing EADs) and the substrate (by enhancing dispersion of repolarization). Numerous drugs have such effects, including class III

antiarrhythmics like sotalol. Several drugs had been recognised to inhibit I_{Kr} or hERG current; for that reason, it is essential in terms of cardiac safety to investigate drug candidates in drug development for hERG current-blocking properties. It should be accentuated that several drugs can cause repolarization abnormality-related arrhythmias without obvious repolarization elongation, however, by attenuating repolarization reserve. Therefore, the effects of drug on other repolarizing currents naming I_{Ks}, I_{K1}, and I_{to} should also take into consideration to eliminate any potential proarrhythmic complexities in the development of novel compounds.

The problem of drug-induced arrhythmia appealed substantial recognition after the reports from two landmarks, large-scale clinical trials, CAST and SWORD. Generally, these trials determine that an empirical strategy to treat arrhythmia is likely to be irrational, as the potential agent that eliminates one arrhythmic mechanism may at the same time aggravate others. For instance, flecainide may reduce arrhythmia by decreasing ectopic activity, however, facilitates arrhythmia via conduction slowing. Sotalol, while exhibiting antiarrhythmic effects due to prolonged refractoriness, may nonetheless facilitates arrhythmogenic EADs. Moreover, there are ample evidence that numerous non–cardiovascular drugs, such as cannabis, cannabinoids, macrolide antibiotics, antihistamines, gastrointestinal or central nervous system drugs can cause arrhythmias as side effects although with lower incidence.

Aims of the study

The aim of the presents study was to investigate the antiarrhythmic and proarrhythmic potential of some pharmacological agents. Therefore, in the present study we studied certain drugs which effect the electrophysiology of the heart such as: Mexiletine-like cellular electrophysiological effects of GS967; Cellular antiarrhythmic and cardiac electrophysiological effects of desethylamiodarone; and, the electrophysiological effects of cannabidiol and quinidine & flecainide.

2. MATERIALS AND METHODS

Adult beagle dogs of either sex weighing 10-15 kg and age 13-27 months (obtained from Asotthalom Hungary, a certified (approval number: XXXV/2018) animal breeder for experimental purposes, were used for the experiments. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (USA NIH publication NO 85-23, revised 1996) and followed to the directive 2010/63/EU of the European Parliament. The Ethical Committee for the protection of Animals in Research of the University of Szeged, Szeged, Hungary (Approval numbers: I-74-15-2017 and I-74-24-2017) and the Department of Animal Health & Food Control of the Ministry of Agriculture and Rural Development Authority (approval numbers XIII3330/2017 and XIII/3331/2017) has approved the protocols.

Recording of APs from multicellular preparations (Conventional Microelectrode Technique)

The AP measurements were recorded in right ventricular muscle and Purkinje fiber from multicellular preparations isolated from dog and/or rabbit hearts using conventional microelectrode techniques as discussed previously in detail. Resting potential (RP), action potential amplitude (APA), maximum upstroke velocity (V^{+}_{max}), and action potential duration measured at 90% of repolarization (APD_{90}) were determined pre and post drug application in cardiac preparation. To investigate the cycle-length (CL) dependency and offset or onset kinetics, the following types of stimulation were applied in the series of the experiments: stimulation with a constant CL of 1000 ms; stimulation with different constant CLs of 300 to 5000 ms range. Following equilibration at 1000 ms CL, the CL was successively varied between 300 and 5000 ms. At each CL the 20th AP was recorded, and the CL was then changed. Under these conditions a quasi-steady-state rate-dependence could promptly be obtained. Restitution kinetics of the maximum rate of depolarization/maximum upstroke velocity (V^{+}_{max}) is considered as the indicator of the offset time constant. To determine the recovery/offset kinetics of V^{+}_{max} and restitution kinetics of APD_{90} (APD_{90} restitution), the preparations were paced using a train of 20 basic stimuli (S1) delivered at a BCL of 1000 ms. An extra test action potential ‘AP’ (S2) was provoked after every 20^{th} basic S1 beat by using single test pulses (S2) in a preparation

driven at a BCL of 1000 ms. The S1–S2 coupling interval was increased progressively from the end of the refractory period. In this way, each 20th basic AP was followed by a single extra AP occurring at gradually increasing diastolic intervals (DI). The DI was the time from APD_{90} of the last basic beat of the train to the upstroke of the extra AP. Restitution curves were created by plotting the V^{+}_{max} and APD_{90} of each extra AP as a function of the respective DI and data were fitted to a single exponential function. Onset kinetics of drug action on V^{+}_{max} were determined by stimulating the preparation at a 400 ms BCL train following a 1-min period of rest and the first 40 APs were recorded, data were plotted against the number of the analyzed AP within the train. Maximum care was taken to sustain the same impalement throughout the whole and each experiment. In case of displacement of impalement, adjustment was made, and the experiment continued if the AP characteristics of the re-adjusted impalement not deviated by more than 5 % of the previous measurement. The effects of compounds were evaluated at the specified concentrations, after the application of each compound until 40-60 min elapsed, in a cumulative manner. Compounds were purchased from Sigma/Merck for all experiments.

Recording of transmembrane ionic currents from single cardiomyocytes

Measurement of late sodium current (I_{NaL})

Conventional voltage clamp experiments, using rectangular command pulses, were performed to study the effects of test compounds on I_{NaL} at stable test potentials. In brief, the sodium current was activated by 2 s-long depolarizing voltage pulses to -20 mV from the holding potential of -120 mV with pulsing CL of 5 s. After 5–7 min, incubation with the drug the external solution was replaced by that containing 20 μM TTX. TTX at this concentration completely blocks the I_{NaL}. The total amount of I_{NaL} was determined by pharmacological subtraction performed by a final superfusion with 20 μM TTX as TTX-sensitive current. The amplitude of I_{NaL} was evaluated at 50 ms after beginning the pulse. To determine the current integral “the charge carried by the current with the exclusion of the initial 20 ms”, the initial 20 ms was omitted from evaluation in order to reduce the contribution of I_{NaP}.

3. RESULTS AND DISCUSSION

3.1 ANTIARRHYTHMIC DRUG EFFECTS

Effects of mexiletine on I_{NaL} using the whole cell configuration of the patch clamp technique (voltage clamp)

Initially, the effect of mexiletine on I_{NaL} was investigated under conventional voltage clamp conditions with 2 s duration depolarization (from holding potential of −120 mV to −20 mV). 40 µM mexiletine significantly declined the density of I_{NaL}, recorded at 50 ms after the onset of the pulse. The reduction in the density of I_{NaL} in 12 myocytes studied was 59.1 ± 1.8% (from −0.385 ± 0.036 to −0.156 ± 0.014 A/F). Comparable results were achieved with the current integrals. As determined from the same experiments, the current integrals were significantly reduced by 63.3 ± 2.7% (from −76.4 ± 7.6 to −26.7 ± 2.6 mC/F) (The results are presented in Roman I article).

Frequency-dependent and recovery kinetic effects of mexiletine on action potential upstroke (V^+_{max})

After evaluating the effect on I_{NaL}, the action of mexiletine on peak fast sodium current (I_{NaP}) was investigated. It is well known that the direct measurement of cardiac I_{NaP} is challenging at 37 °C, therefore, the maximum velocity of depolarization ($V^+_{max.}$) during the AP upstroke was used as an estimate measure of I_{NaP}. Keeping in view the better physiological condition (such as higher stability) of multicellular cardiac preparations, the right ventricular muscles were used to perform these experiments using sharp microelectrodes. 40 µM mexiletine markedly and significantly inhibited V^+_{max} at CL shorter than 1 s and exerted only moderate effect at CL longer than 1 s. Moreover, 40 µM mexiletine significantly affects the offset kinetics of V^+_{max}. The time constant of recovery of V^+_{max}, recorded at 1 Hz stimulation, was 289 ms for mexiletine.

Onset kinetic effects of mexiletine on V^+_{max}

Following the offset kinetics, the onset kinetics of V^+_{max} -block was investigated in ventricular muscle tissues at 400 ms constant stimulation rate following a one-minute rest

and then recorded the first 40 APs. The onset rate constant was 2.6 AP for 40 µM mexiletine.

Frequency-dependent effects of mexiletine on action potential duration (APD)

The effect of mexiletine was studied on APD_{90} in ventricular muscle preparations representing subendocardial origin using the conventional sharp microelectrode technique. At BCL shorter than 1 s, mexiletine did not change the APD significantly, but 40 µM mexiletine shortened APD_{90} at the longer constant CL than 1.5 s.

Restitution kinetic effects of mexiletine on APD

The restitution of APD following mexiletine effect was measured in right ventricular muscles by the double pulse protocol (S1-S2) as described in methods section. 40 µM mexiletine increased refractoriness but the time constant of restitution of APD_{90} recorded following a constant 1 Hz stimulation could not be determined properly after mexiletine application due to the increase of the effective refractory period (ERP).

Effects of GS967 on I_{NaL} using patch clamp technique

Similar to that of mexiletine, the effect of GS967 was studied on I_{NaL} as per protocol discussed above. 1 µM GS967 comparatively and significantly decreased the density of I_{NaL} from −0.313 ± 0.05 to −0.062 ± 0.01 A/F (reduction of 80.4 ± 2.2%, n=6). Similarly, the current integrals were significantly decreased by 79.0 ± 3.1% (from−69.1 ± 7.9 to −15.4 ± 3.9 mC/F).

Frequency-dependent and recovery kinetic effects of GS967 on V^{+}_{max}

The similar effect of GS967 on I_{NaP} was investigated by using the above mentioned protocols. The inhibitory effect of GS967 on V^{+}_{max} was significant only at the shortest CLs of 0.3 s and 0.4 s as shown in. This frequency-dependent effect can well be described by the faster offset and onset kinetics of GS967. The time constant of recovery of V^{+}_{max} at 1 Hz stimulation was 110 ms for GS967, this accounts for almost three times shorter time constant of recovery of V^{+}_{max} than mexiletine.

Onset kinetic effects of GS967 on V^{+}_{max}

Further, to investigate the effect of GS967 on the onset kinetics, we recorded the effect of 1 μM GS967 on V^{+}_{max} -block at 2.5 HZ stimulation rate similar to that of mexiletine. The onset rate constant was 5.3 AP for 1 μM GS967.

Frequency-dependent effects of GS967 on APD

In ventricular muscle preparations, GS967 did not significantly change the APD_{90} at CLs of shorter than 2 s stimulation. However, 1 μM GS967 significantly shortened APD_{90} at stimulation CL longer than 1.5 s.

Restitution kinetic effects of GS967 on APD

Similar protocols were followed, as for mexiletine, to study the effect of GS967 on the restitution of APD_{90}. 1 μM GS967 slowed the kinetics of restitution. The time constant of recovery of APD_{90} recorded following a constant 1 Hz stimulation was 12.8 ± 1.1 ms for control and 18.3 ± 2.3 ms for 1 μM GS967.

Effects of desethylamiodarone (DEA) on the dispersion of repolarization in the ventricle

DEA lengthened APD in ventricle but not in Purkinje fibers as contrary to the effects of Class III antiarrhythmic drugs. In our results, the dispersion of repolarization (differences in APD between Purkinje and ventricular tissue) was compared after chronic DEA 50 μM and acute sotalol (3 and 10 μM) applications. DEA and sotalol distinctly and significantly lengthened APD in ventricular muscles, however, the dispersion of repolarization was markedly higher in sotalol-treated preparations than chronic DEA administration. This effect suggests less proarrhythmogenic potential of DEA vs. sotalol (The results are presented in Roman III article).

Effects of acute DEA administration on dog ventricular APs (APD and V^{+}_{max})

In several experiments, the effects of acute 10 μM DEA application on cardiac APs also were studied. In dog right ventricular preparations 10 μM acute DEA exerted a mild but not reverse rate dependent APD_{90} prolongation and significant V^{+}_{max} inhibition at short cycle lengths (CL<700ms).

Effects of acute DEA administration on ouabain-induced delayed afterdepolarizations (DADs) in dog Purkinje fibers

Further, delayed afterdepolarizations (DAD) were induced in Purkinje fibers pretreated with 0.3 μM ouabain. 10 μM DEA application abolished the DADs in all six out of six experiments. These results are in agreement with the previously reported results from our lab with similar acute administration of 10 μM AMIO (Varro et al., 2001).

Class 1 antiarrhythmic effects of flecainide on I_{NaL}

3 μM flecainide significantly reduced the density of I_{NaL} from −0.311 ± 0.05 to −0.114 ± 0.01 A/F (reduction of 63.2 ± 3.0%, n=4). Similarly, the current integrals were significantly decreased by 56.3 ± 4.6% (from −57.9 ± 9.7 to −24.7 ± 3.9 mC/F).

Frequency-dependent effects of flecainide on APD

The frequency dependent actions of flecainide (1, 3, 10 μM) on APD_{90}. Flecainide, however not significant, lengthened APD_{90} only at faster stimulation frequencies shorter than CL of 500ms.

Restitution kinetic effects of flecainide on APD

The restitution of APD_{90} following flecainide application was measured in ventricular muscle by the same protocols as described above. The time constant of restitution of APD_{90} recorded in control following a constant 1 Hz stimulation was 7.7 ± 1.2 ms, but, since the restitution curve after flecainide application did not show single exponential behaviour and was not determined. It has to be emphasized that APDs of early extrasystoles were significantly and markedly increased after flecainide administration,

Class 1 antiarrhythmic effects of quinidine on I_{NaL}

10 μM quinidine significantly reduced the density of I_{NaL} from −0.416 ± 0.06 to −0.212 ± 0.03 (reduction of 46 ± 4.3%, n=8). Similarly, the current integrals were significantly decreased by 41.4 ± 5% (from −83.1 ± 13 to −47.6 ± 8.3 mC/F).

Effects of quinidine on APD restitution kinetic

The restitution of APD_{90} following quinidine application was measured in ventricular muscle by the same protocols as described above. Quinidine slowed down the kinetics of restitution at all studied concentrations. Quinidine increased APD_{90} (and refractory period) throughout the entire restitution curve. The time constant of restitution of APD_{90} recorded following a constant 1 Hz stimulation was 32.3 ± 2.1 ms for quinidine 20 μM. vs. 5.8 ± 1.4 ms for control.

3.2 PROARRHYTHMIC DRUG EFFECTS

Proarrhythmic potential of sodium channel blockers

Frequency-dependent effects of flecainide on V^+_{max}

Flecainide reduced V^+_{max} in a concentration-**,** and frequency-dependent way, maximum reduction occurred at the faster frequency rates (shorter CLs). Similarly, the effect of flecainide on the recovery kinetics of V^+_{max} was rate-dependent, with greater depression of V^+_{max} occurring at faster pacing rates. The time constant of recovery of V^+_{max}, determined following a constant 1 Hz stimulation, was 9.8 ± 4.0 s for flecainide 10 μM vs. 15.6 ± 0.9 ms for control.

Onset kinetic effects of flecainide on V^+_{max}

The onset kinetics of this use-dependent V^+_{max} -block after flecainide application was dose-dependent, relatively faster at higher concentration, characterized by beat constants of 11.0 ± 0.8 for flecainide 10 μM at 400 ms CL stimulation.

Frequency-dependent effects of quinidine on V^+_{max}

Like flecainide, quinidine shows the concentration-**,** and rate-dependence blockade of V^+_{max}. V^+_{max} depression was markedly significant at faster stimulation rates but it was present at wide range of CLs. The time constant of recovery of V^+_{max}, determined following a constant 1 Hz stimulation, was 4.0 ± 0.4 s for quinidine 20 μM vs. 24.0 ± 2.3 ms for control.

Onset kinetic effects of quinidine on V^{+}_{max}

Similarly, the onset kinetics of this concentration-, and use-dependent V^{+}_{max} -block after quinidine application was faster with higher concentration, characterized by the onset rate constant of 4.1 ± 0.5 AP for quinidine 20 μM at 400 ms CL stimulation.

Frequency-dependent effects of quinidine on APD

Quinidine shows the concentration-, and rate-dependent (reverse) action on APD. Quinidine lengthened APD in the entire range of frequencies while showing a more pronounced lengthening effect on APD at slower stimulation frequencies.

Proarrhythmic potential of cannabinoids

The cardiac cellular electrophysiological effect of CBD on APs in rabbit and dog ventricular preparations

CBD lengthened APD_{90} significantly at the concentration of 5 μM without changing any other AP parameters significantly such as APA, V^{+}_{max}, RMP. This effect on repolarization in rabbit and dog ventricular preparation can be best explained by the multiple effects CBD exerts on various transmembrane ionic currents underlying the AP (The results are presented in Roman III article).

CLINICAL IMPLICATIONS

Drugs from numerous therapeutic classes may induce or aggravate a variety of arrhythmias. It is important to be aware of the drugs which might cause arrhythmias and specifically about drug-induced arrhythmias. Attention towards the risk factors, in case of some drug-induced arrhythmias, can assist in prevention and risk reduction. Although, the mechanisms behind the arrhythmias associated with certain drugs are largely unknown. Therefore, it is needed to conduct more research in this area to further provide data to properly define the overall occurrence of certain drug-induced arrhythmias, its underlying mechanisms, the standard methods to decrease the risk and enhance the awareness among the clinicians and the patients. It is also important to consider the possibility that a patient's arrhythmia could be drug-induced. Furthermore, it becomes more important during electrophysiological characterization of an agent on the cardiac ion channels to test the normal-, and reverse rate-dependent nature of the cardioactive drugs action by utilizing a wide range of stimulation frequencies. Moreover, in order to translate the date from animal experiments to clinical relevant data, larger mammals like dogs should be considered as there is large differences between the normal heart rate in human and the faster spontaneous frequencies of smaller laboratory animals such as mice, rats, guinea pigs and rabbits.

THE MOST IMPORTANT NEW RESULTS:

The most important new results of my PhD thesis are as follows:

1. There are important differences in the antiarrhythmic and proarrhythmic potentials between antiarrhythmics drugs belonging into the same class.
2. GS967 has both I_{NaP} and I_{NaL} inhibiting properties, which contrasts previous statements that this compound represents new class of so called "I_{NaL} specific inhibitor".
3. I_{Na} inhibitors blocking I_{Na} with fast onset and offset kinetics (Mexiletine and GS967) of block are beneficial since they have less proarrhythmic potential than those having slow onset and offset kinetics of I_{Na} inhibition (Flecainide and Quinidine).
4. Flecainide have specific APD lengthening effect on the APD of early extrasystoles which effect can be an unrecognized cellular antiarrhythmic property.

5. DEA which is the main metabolite of amiodarone has beneficial effect by decreasing depression of repolarization between ventricle and Purkinje fibers.
6. CBD has a previously unrecognized mild but discernible ventricular APD lengthening effect which may represent increased risk of proarrhythmia in the clinical practice.

CONCLUSION

Contrary to other class 1 agents, class 1a and 1c agents slowly dissociate from the Na^+ channel and hence remarkably delay ventricular conduction and therefore in the presence of arrhythmic substrate could further enhance this proarrhythmic risk. These findings suggest that the frequency dependence, restitution kinetics and onset kinetics are important electrophysiological determinants which can discriminate Na^+ channel blockers with proarrhythmic and antiarrhythmic potential. Moreover, the current data can suggest to the clinicians that probably compounds which does not block impulse conduction at normal heart rate and shortens APD because of the inhibition of late sodium current and have a fast recovery such as mexiletine and GS967 compounds may be beneficial for future drug development. Also the interesting marked effect of APD prolongation on the early extrasystole observed after flecainide application would be probably useful antiarrhythmic property. Furthermore, the absence of an increase in dispersion of ventricular repolarization with DEA correlates with its clinically observed lower incidence of proarrhythmia. In addition, the physicians should be aware in recognizing of the aforementioned deleterious effects of compounds that affect the repolarization reserve in co-morbid or polypharmacy patient.

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