Dynamic regulation of the late sodium current in ventricular cardiomyocytes

project closing report

In the project the so-called „late sodium current" I(Na,late) was examined in ventricular cardiac preparations obtained from canine, guinea pig, rabbit, and human tissues.

RESULTS

(1) Canine vs guinea pig I(Na,late)

Canine I(Na,late) displayed a "decrescendo" profile, i.e. its amplitude decreased monotonically during the time course of the AP, in contrast to guinea pig ventricular myocytes, where the amplitude of the current was increasing during the plateau and declined only on terminal repolarization ("crescendo profile"). These differences are also reflected by the current-voltage relations (phase-plane trajectories) obtained under the APs in canine and guinea pig cells. To demonstrate the profile of I(Na,late) quantitatively the TTX-sensitive current density was determined at 20%, 50% and 100% of the AP duration at 90% of repolarization (APD90). Accordingly, the density of I(Na,late) at 20% APD90 was −0.52±0.09 A/F in dog versus the −0.21±0.05 A/F in guinea pig ($p<0.05$), while at 100% APD90 I(Na,late) had a greater density in guinea pig than dog (−0.78±0.07 versus −0.03±0.02 A/F, $p<0.05$). At 50% APD90 the densities were not significantly different in the two species. Although the charge carried by I(Na,late) was higher in guinea pig than in dog (86.2±10.8 mC/F, n=20/9 versus 67.1±9.2 mC/F, n=7/4, $p<0.05$) these current integrals were in a similar range.

AP configuration influences current profiles under APVC conditions, therefore we attempted to convert the decrescendo type canine I(Na,late) into a „crescendo" profile, characteristic of guinea pig myocytes. Therefore, duration-matched canonic guinea pig APs and voltage ramps resembling the ramp-like plateau of guinea pig cells were applied as command signals to canine cells. The ramp started from −80 mV, spent 10 ms at +40 mV and decayed to −20 mV during 200 ms. None of these interventions were capable to convert the canine decrescendo current profile to a crescendo guinea pig-like I(Na,late) profile. The current integrals were also similar in canine cells independently of the canine or guinea pig origin of the command AP (63.1±9.2 mC/F, n=9/5 versus 65.4±10.7 mC/F, n=6/5, N.S.).

We compared kinetic properties of I(Na,late) in canine and guinea pig ventricular cells using conventional voltage clamp. The 20 μM TTX-sensitive currents were similarly shaped in all types of myocytes when the membrane potential was switched from the holding potential of -120 mV to the test potential of −20 mV in the presence of 1 μM nisoldipine. When the decay of TTX-sensitive current was fitted to a monoexponential function the inactivation time constants were 60±3 ms (n=13/7) in canine and 155±16 ms (n=5/3) in guinea pig cells. The corresponding current densities (obtained by monoexponential fitting) were −0.56±0.04 A/F (in canine) and −0.54±0.14 A/F (in guinea pig), which values were not significantly different.

In conclusion, it is very likely that the differences in the shape of canine and guinea pig I(Na,late) under the AP is determined by the different inactivation kinetics of the sodium channels that generate I(Na,late) in the two species. The decay time constant is much longer in guinea pig, thus the inactivation can be slow enough to leave a significant portion of Na+ cannels open by the time of terminal repolarization. Consequently, the increasing inward driving force acting on the Na+ ions could increase the amplitude of I(Na,late) during the monotonic slow repolarization in guinea pig cells. There might be differences in sodium channel isoforms that might underlie the differences observed in the macroscopic current.

(2) Effects of GS-458967 (GS967) vs mexiletine on I(Na,late)

Effects of GS967 and mexiletine on I(Na,late) were studied under conventional voltage clamp conditions by applying 2 s duration depolarizations to −20 mV from the holding potential of −120 mV. 1 μM GS967 significantly reduced the density of INaL, measured at 50 ms after the beginning of the pulse (from -0.313 ± 0.05 to -0.062 ± 0.01 A/F, corresponding to an 80% reduction. For comparison, this parameter was also significantly decreased by 40 μM mexiletine from -0.385 ± 0.036 to -0.156 ± 0.014 A/F (reduction of about 60%). Similar results were obtained when comparing the current integrals.

Both GS967 and mexiletine caused a significant leftward shift in the steady-state inactivation curve of I(Na,late). The V0.5 value was shifted by −17.2 and −13.5 mV in the presence of GS967 and mexiletine, respectively.

1 μM GS967 and 40 μM mexiletine dissected inward I(Na,late) profiles having current densities (measured at 50% of APD90) of -0.37 ± 0.07 and -0.28 ± 0.03 A/F, and current integrals of -56.7 ± 9.1 and -46.6 ± 5.5 mC/F, respectively. These differences were not significant statistically.

40 μM mexiletine significantly reduced V+max in the entire frequency range applied under steady-state conditions, while this effect of GS967 was significant only at the shortest applied cycle lengths of 0.3 and 0.4 s. This difference can well be explained by the faster offset kinetics of GS967. The time constant of recovery of V+max, determined following a constant 1 Hz stimulation was 110 ms for GS967, while almost three times longer, 289 ms for mexiletine. The onset kinetics of V+max block was studied by application of a constant stimulation rate at 2.5 Hz and the initial 40 APs were recorded. The onset rate constant was 5.3 AP for 1 μM GS967 and 2.6 AP for 40 μM mexiletine.

(3) I(Na,late) is enhanced by baseline CaMKII activity in canine left ventricular myocytes

Under action potential voltage clamp conditions, 1 μM GS967 and 10 μM TTX dissected similar inward current profiles in canine ventricular cells. These concentrations were chosen because the densities, measured at 50% of APD90 (-0.42±0.03 versus -0.40±0.04 A/F) and integrals (-68±5 versus -61±6 mC/F) of the dissected currents were largely comparable in size.

Ca2+-sensitivity of I(Na,late) was studied by blocking ICaL with 1 μM nisoldipine in order to reduce the Ca2+ entry into the myocytes. I(Na,late) was smaller following nisoldipine pretreatment than under control conditions. There could be two possible explanations for this behavior. First, GS967 might also suppresses ICaL at the applied concentration. In this case, if GS967 is used without L-type calcium channel blockade, the GS967-sensitive current will be contaminated with a small fraction of ICaL. Second, I(Na,late) could be modulated by changes of intracellular Ca2+ concentration. In this case, GS will be smaller, because nisoldipine pretreatment reduces [Ca2+]i.

We examined whether GS inhibited L-type calcium current under conventional voltage-clamp conditions. Our results showed that there were no significant differences in the examined parameters of the L-type calcium current (maximum current density, current density at 50 ms after the peak, and the charge carried by the current).

To support the role of [Ca2+]i in the regulation of I(Na,late), the cytosolic Ca2+ was reduced by 10 mM BAPTA added to the pipette solution. In the presence of intracellular BAPTA, both I(Na,late) density, measured at 50% of APD90 (-0.30±0.03 A/F vs -0.42±0.03 A/F), and the current integral (-46.7±5.2 vs -68±5 mC/F) were significantly lower than obtained under control conditions.

To test the hypothesis of Ca2+-dependent augmentation of I(Na,late), we studied the role of CaMKII, the most likely candidate to mediate Ca2+-dependent effects on I(Na,late). In these experiments the pipette solution contained inhibitors of CaMKII, either 1 μM KN-93 or 0.5 μM autocamtide-2-related inhibitor peptide (AIP). I(Na,late) densities, measured at 50% of APD90 were significantly smaller when CaMKII was inhibited compared to control conditions. Current integrals were also smaller with KN-93 and AIP; however, these changes did not reach the level of statistical significance ($p = 0.2$, and $p = 0.09$, respectively).

In conclusion, I(Na,late) (defined as GS-sensitive current) was significantly smaller whenever the L-type calcium current was inhibited, the ic. calcium was chelated with BAPTA, or if CaMKII was inhibited either by KN-93 or by autocamtide-2-related inhibitor peptide (AIP). These results show that I(Na,late) is enhanced by baseline CaMKII activity in canine left ventricular myocytes.

(4) Effects of isoproterenol on canine I(Na,late)

Superfusing the cells with 10 nM isoproterenol for 15 min increased the density and the integral of I(Na,late) with preservation of the original decrescendo profile. This stimulation was prominent at the early phase of I(Na,late) (at 20% APD: −0.69±0.09

versus −0.52±0.09 A/F and at 50% APD: −0.55±0.11 versus −0.34±0.06 A/F; integrals: 87.4±13.3 versus 67.1±9.2 mC/F; n=5/3 and n=7/4 respectively; $p<0.05$ for each parameter).
In order to elucidate the exact intracellular signaling mechanism of how β-adrenergic stimulation leads to increased I(Na,late) further studies are necessary.

(5) Late Na+ currents and their conductances under action potential voltage clamp conditions in canine, rabbit, and guinea pig ventricular myocytes

We recorded I(Na,late) profiles obtained in canine, rabbit and guinea pig ventricular myocytes under self APVC conditions; i.e. the cell's own AP was used as a command pulse in all cases. G(Na,late) profiles were calculated according to the Nernst potential of Na^+.
The density of I(Na,late) was relatively constant during the AP plateau phase and decreased only along the terminal repolarization of the AP in canine and rabbit myocytes, while G(Na,late) decreased monotonically in these cells during the AP. On the contrary, in guinea pig myocytes I(Na,late) increased monotonically, while G(Na,late) remained largely unchanged during the AP. The current-voltage relationships were also different by shape in guinea pig comparing to dog or rabbit.

The rate of decay of G(Na,late) was estimated as the reduction of G(Na,late) between the time spent between 20% and 80% of APD90, normalized to G(Na,late) that measured at the time of 20% of APD90 ("decay factor": defined as (G20% - G80%) / G20%). Indeed, this decay factor was significantly less in guinea pig (−0.07±0.16, n=18) than in canine (0.46±0.06, n=15) or rabbit (0.60±0.04, n=6) myocytes. Although the decay of G(Na,late) was seemingly faster in rabbits than in dogs, this difference was not significant statistically. The charge carried by I(Na,late) (i.e. the integrals) were similar in dog (−64.2±6 mC/F) and rabbit (−66.5±14.6 mC/F), but both of them were significantly less than the integral obtained in guinea pig cells (−94.6±9.6 mC/F).

Current profiles under APVC conditions are affected by the shape of the AP voltage pulse. Therefore time-matched canonic rabbit and guinea pig APs were delivered to canine myocytes to answer the question that the marked differences seen in the G(Na,late) profiles in guinea pig versus dog and rabbit are related to differences in AP configuration or are genuine interspecies differences in Na+ channel gating. Despite applying rabbit or guinea pig command Aps on canine cells, the shape of I(Na,late) remained unchanged, i.e. showed the "decrescendo" profile – the characteristic of canine I(Na,late). Importantly, the current integrals were similar in the canine cells independent of whether canine, rabbit or guinea pig command APs were applied (canine AP: −48.5±5 mC/F, n=19; guinea pig AP: −49.9±8.4 mC/F, n=8; rabbit AP: −57.0±22.2 mC/F, n=4; N.S.).

To study the role of [Ca2+]i in the regulation of I(Na,late), the cytosolic Ca2+ was reduced by applying either 1 μM nisoldipine in the bathing medium or 10 mM BAPTA in the pipette solution. In the latter case, measurements started 10 min after rupturing the seal to let the Ca2+ chelator BAPTA equilibrate between the pipette solution and

the intracellular space. The amplitude of I(Na,late) was reduced when the intracellular Ca2+ concentration was decreased by nisoldipine (−330±30 mA/F vs −457±38 mA/F at 20% APD90, and −282±38 vs −412±37 mA/F at 50% APD90, p< 0.05, n=19 vs n=15), although at 80% APD90 the difference was not significant. The effect of BAPTA was significant at each segment of APD (−277±44 vs −457±38 mA/F at 20%, −306±36 vs −412±37 at 50%, and −223±32 vs −284±34 mA/F at 80% APD90, p<0.05, n=11 vs n=15). When calculating conductances, G(Na,late) was reduced by nisoldipine, similarly to reduction of I(Na,late) (5.0±0.5 mS/F vs 6.9±0.7 mS/F at 20% APD90, and 4.0±0.5 vs 5.9±0.6 mS/F at 50% APD90), but the reduction of G(Na,late) was significant only at 80% APD90 (2.0±0.3 mS/F vs 3.5±0.4 mS/F). In other words, nisoldipine decreased G(Na,late) more prominently at the initial, while BAPTA at the later segment of the AP. Current integrals, however, were significantly reduced by both nisoldipine and BAPTA (−48.5±5 mC/F vs −63.9±6 mC/F and −46.7±5 vs −63.9±6 mC/F, respectively).

The toxin of Anemonia sulcata (Anemone toxin II, ATX-II) induces a current in cardiac tissues closely resembling I(Na,late) by inhibiting the fast inactivation mechanism of Na+ channels. Therefore, the effect of 10 nM ATX-II in guinea pig and 1 nM ATX-II in canine myocytes were studied. When 10 nM ATX-II was applied for 3 min, canine cells usually produced early afterdepolarizations (data not shown). To prevent these, canine myocytes were treated with only 1 nM ATX-II. This demonstrates that the sodium channels in canine myocytes are more sensitive to ATX-II exposure than the channels in guinea pig cells. ATX-II lengthened the AP and increased the amplitude of I(Na,late) in both species. Since I(Na,late) was recorded in the absence and in the presence of ATX-II in different sets of experiments, only the average I(Na,late) and G(Na,late) profiles (without SEM values) could be compared. Although the ATX-II induced currents were not identical to native I(Na,late), their profiles were similar in shape to the profiles of the native I(Na,late) in both species. The ATX-II induced current displayed a “decrescendo” profile in canine and “crescendo” profile in guinea pig myocytes – just like their respective native I(Na,late) profiles. However, in contrast to the native G(Na,late) profile, which was largely constant during the AP plateau in guinea pig, the ATX-II induced conductance displayed a marked increasing tendency during the guinea pig AP. Accordingly, the decay factor estimated for G(Na,late) in the presence and in the absence of ATX-II was similar (0.54±0.06, n=6 and 0.46±0.06, n=15, respectively, N.S.) in canine myocytes. In guinea pigs, the decay factor became significantly more negative in the presence of ATX-II than measured under control conditions (−0.95±0.81, n=4 vs −0.07±0.16, n=18, p<0.05). These results demonstrate that the behavior of the ATX-II-induced conductance is markedly different from the native G(Na,late) in guinea pig myocytes.

COMMUNICATION OF THE RESULTS

Our research results have been reported in the form of in extenso publications. In eight of these publications the PI of the current project is either the first-, or the last author.
One further in extenso publication has already been accepted for publication (Balázs Horváth et al. “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" accepted for publication in *Pharmaceuticals*)
In connection with the topic, we actively participated in several domestic (Hungarian Society of Cardiologists, Hungarian Physiological Society) and international (European Heart Rhythm Association, European Working Group on Cardiac Cellular Electrophysiology, International Academy of Cardiovascular Sciences) conferences, presenting lectures or posters.

FUTURE PLANS

Some of our unpublished results will be used later for a publication examining the adrenergic regulation of I(Na,late) in more details.