Closing report for project NKFIH PD-120794

Conventional sharp microelectrode measurements were carried out to evaluate how the blockade of the late sodium current (INa,late) and the current of TRPM4 channels (ITRPM4) affect various parameters of the action potential (AP).

Late sodium current

The blockade of INa,late (1 μM GS) significantly influenced the studied AP parameters. AP duration at 90% of repolarization (APD90) was shortened by 14% and the plateau potential measured in the middle of the plateau phase (plateau50) was decreased by 6 mV. AP amplitude (APA) and maximal rate of depolarization (dV/dtmax) decreased significantly as well, most likely due to a small early sodium current blocking effect of 1 μM GS. The blockade of INa,late decreased the short-term variability of APD90 by 18%, although this effect did not reach statistical significance.

Frequency dependent effects of INa,late blockade by 1 μM GS was studied in the range of 300, 500, 700, 1000 and 2000 ms pacing cycle lengths (PCL). The blockade of INa,late showed reverse frequency dependent effects on APD90. 1 μM GS decreased dV/dtmax with about 110-120 V/s at all PCLs, this effect seemed to be independent of PCL. 1 μM GS decreased APA significantly at all PCL studied, having more pronounced effects at 300 ms.

To study the profile of INa,late under an actual AP, the “action potential voltage-clamp” (APVC) technique of the whole cell configuration of patch clamp was used. We used a previously chosen “canonic” midmyocardial canine AP as the voltage command, in order to abolish the possibility of differences in the current traces due to different voltage commands. About 10-15 milliseconds after the AP peak, the canine INa,late starts with a current density around 0.5 A/F, has about the same density until around the middle of the plateau phase, and after that it gradually decreases. According to the 6 cells studied, the total charge carried by the current was 0.061±0.008 C/F.

TRPM4 current

The effect of 9-phenanthrol on AP morphology was first tested in concentrations of 1, 3, 10 and 30 μM at the stimulation rate of 1 Hz. 9-phenanthrol caused a concentration-dependent depression of plateau50 potential. This effect was significant from 3 μM and was reversible upon washout. The maximal rate of depolarization was also significantly decreased from the concentration of 3 μM, but it was only partially reversible upon washout. 30 μM 9-phenanthrol significantly decreased the action potential amplitude.

The slope of early repolarization (phase 1) was significantly reduced by 9-phenanthrol. This was the strongest effect of the drug on action potential configuration: it was significant from 3 μM concentration and was fully reversible upon washout. The rate of terminal repolarization was reduced significantly by 10 and 30 μM 9-phenanthrol however it was only partially reverted by washout.

The effect of 9-phenanthrol on APD90 showed no significant change up to 10 μM concentration, but APD90 began to increase in the presence of 30 μM which effect progressively continued during the washout.

Based on the effects of 9-phenanthrol on AP morphology, 9-phenanthrol seems to be a rather unspecific compound. Therefore in the future, we are planning to investigate the effects of 9-phenanthrol on the major cardiac ventricular ionic currents by conventional voltage clamp experiments. These experiments are necessary to see whether we will be able to use 9-phenanthrol as a specific blocker to achieve one of the major goals of the projects: to visualize ITRPM4 under the AP by APVC technique. If 9-phenanthrol proves to block other channels besides TRPM4 we will need to search for a different drug candidate (that specifically blocks TRPM4 channels) to be used in our APVC experiments.

Effects of BAPTA-AM on action potential morphology

We were planning to buffer intracellular calcium ([Ca2+]i) in some of our forthcoming experiments by using the cell-permeant acetoxy-methylester form of BAPTA (BAPTA-AM). Therefore we investigated the time-dependent actions of extracellularly applied BAPTA-AM on the action potential configuration of our experimental model cells.

Exposing the cells to 5 μM BAPTA-AM caused an initial rapid rise in APD90, followed by a slower, gradually developing AP lengthening effect. The changes in APD90 were accompanied by characteristic changes in action potential morphology, since in the presence of BAPTA-AM (presumably the reduction of [Ca2+]i shifted the plateau potential to more positive voltages.

Next, the effect of BAPTA-AM was studied under conditions when the L-type calcium current (ICa,L) magnitude was manipulated. In the presence of the Ca2+-channel blocker nisoldipine the BAPTA-AM-induced APD-lengthening was negligible (although statistically significant) and only transient since it disappeared after 20 min exposure to BAPTA-AM. In contrast, increasing ICa,L density with BAY K8644 markedly augmented the BAPTA-AM-induced prolongation of the AP.

We have also investigated the effect of BAPTA-AM in the presence of an IKr and IKs blockers (100 nM dofetilide and 1 μM HMR-1556). In the presence of IKs blocker HMR-1556, BAPTA-AM lengthened the AP, whereas upon pretreating the cells with the IKr blocker dofetilide, BAPTA-AM had an APD90 shortening effect. On the other hand, the APD90-lengthening effect of 100 nM dofetilide was significantly reduced in the presence of 5 μM BAPTA-AM. The effect of BAPTA-AM on action potential morphology was sensitive exclusively to the density of IKr, since upon pretreating the cells with 1 μM HMR-1556 BAPTA-AM still exerted its AP lengthening effect.

A possible explanation of the lack of APD lengthening effect of dofetilide in the presence of BAPTA-AM could be that BAPTA-AM itself blocks the IKr current. Therefore, the effect of externally applied BAPTA-AM on IKr was studied using the conventional patch clamp technique. Exposing the cells to 5 μM BAPTA-AM for 5 min decreased the density of IKr to 32±8 % of the control in a partially reversible manner, since the current amplitude returned to 76±5 % of its control value during a 5 min period of washout with BAPTA-AM-free superfusate. As the pipette solution contained 10 mM BAPTA in these experiments, suppression of IKr by BAPTA-AM was independent of intracellular Ca2+ buffering. Based on the IKr blocking properties of BAPTA-AM, we will need to be careful when applying extracellular BAPTA-AM to chelate [Ca2+]i.

The principal investigator has renounced project #120794 effective 31st August 2017.