THE FLOCALIN ACTION ON HEART-SPECIFIC COMBINATION OF K-ATP CHANNELS AND ITS DOSE-RESPONSE

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Activators of K-ATP (potassium ATP sensitive) channels are used as cardioprotectors in case of ischemia. The opening of K-ATP channels leads to hyperpolarization, reduction in contractility and decline in cellular metabolism. Thus it protects cardiomyocytes from damage. Flocalin is the new opener of the K-ATP channels. The presence of the fluorine group made the flocalin less toxic and more stable compared to its predecessor – pinacidil. It is known that flocalin has strong cardioprotective action against ischemia-reperfusion damage in the whole heart [1]. But there are some tissues also having K-ATP channels and the flocalin tissue specificity remains unclear. To investigate this question we developed the expression system which will artificially reproduce the K-ATP channels subunits composition of appropriate tissue. For sufficient comparing of flocalin action on different tissue-specific combinations of the K-ATP channels the estimation of its dose-response and its effective concentration is needed.

The expression system of the K-ATP channels is made as transfected HEK-293 cells with plasmid vectors containing complementary DNA sequences of K-ATP subunits appropriate to a certain tissue. The Kir6.2 and SUR2A combination of subunits is typical for heart tissue. We have found it using RT-PCR (reverse transcription-polymerase chain reaction) approach. Thus to obtain heart-specific expression we have transfected HEK-293 cell line with Kir6.2 and SUR2A subunits coding sequences. On such HEK-293 cells we have measured currents through expressed ion channels and flocalin application effect on it using the electrophysiological approach – patch-clamp.

In our previous investigations we have found that 20 uM flocalin solution activated current of lower amplitude compared to 20 uM of pinacidil. Both types of currents evoked by pinacidil and flocalin were blocked by K-ATP channel blocker – glibenclamide (10 uM) and had appropriate electrophysiological characteristics for K-ATP channels. The flocalin effect on potassium current differs depending on its concentration and is not as same as the pinacidil effect with the same concentration. According to this, it is necessary to define flocalin dose-response. It will help to compare the flocalin action on different tissues.

We measured potassium currents activated by application of 0.5, 0.75, 1,5, 10, and 20 uM flocalin. The effective concentration EC_{50} was about 5.4 uM. The obtained dose-response curve has a more sharp form than the pinacidil one comparing to the literature [2]. The current amplitude increases at low concentration. Therefore we suggest that flocalin is a more potent substance since it induces the same effect with a lower concentration than pinacidil. The less amount of substance is needed to activate the certain number of K-ATP channels.

In conclusion, we suggest that flocalin has no same effectiveness as pinacidil. Due to its stability, less toxicity, and potency the flocalin can be better than pinacidil in pharmacology issues. The data of dose-response will be used for further experiments on expressed compositions of other tissues: pancreas and bladder smooth muscle. Further experiments with flocalin on tissue-specific compositions of K-ATP channels are necessary.

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