A-2

The role of Na^+-Ca^{2+} exchange on calcium activated chloride current in single isolated cardiac myocyte in pulmonary vein of rabbit.

Won Tae Kim, Yoon Jin Lee, Jeong Mi Ha, Han Choe, Yeon Jin Jang, Chun Sik Park, Chae Hun Leem

Department of Physiology, University of Ulsan, College of Medicine

We have shown the Ca^{2+}-activated chloride current is present in cardiac myocyte in rabbit pulmonary vein (Kim et al., 2002). This current amplitude was increased as $[Na^+]_i$ was increased and we suggested this chloride current may be involve in the spontaneous action potential frequency change. Since this current is activated by the increase of intracellular Ca^{2+}, we would like to test what is the inducer of the increase of $[Ca^{2+}]_i$ between a L-type Ca^{2+}-current or a reverse mode of Na^+-Ca^{2+} exchange current. White rabbit (1.5 kg) was used and anesthetized with Ketamin (100 mg/kg). Pulmonary vein (PV) was isolated and sleeve area between left atrium and PV was dissected. Using collagenase (Worthington 0.7 mg/cc), single cardiac myocytes were isolated. In the presence of 15 mM of Na^+, three steps of voltage pulses were applied (holding potential : -40 mV, -80 mV for 50 msec, 30 mV for 5 msec, 10 mV steps from -70 mV to 60 mV). The inward and outward tail current was activated after brief 5 msec prepulse. The outward tail current was blocked by the removal of extracellular chloride substituted by glucuronic acid or by a chloride channel blocker, 5 mM 9-AC. But the inward tail current was still remained even though the amplitude was decreased. The reversal potentials were changed to the direction of the change of chloride equilibrium potential (E_{Cl}) but the shift of equilibrium potential was not enough to match to the theoretical equilibrium potential shift. In the presence of L-type Ca^{2+} channel blocker, nifedipine 1 uM, inward tail currents were greatly reduced but the outward current tail currents were still remained. In the presence of Na^+-Ca^{2+} exchange current blocker, 10 uM KB-R7943, the inward and outward tail currents were blocked almost completely. We tried to test the Ca^{2+} sensitivity of the chloride current with various $[Ca^{2+}]_i$ in pipette solution from 100 nM to 1 uM but we failed to activate Ca^{2+}-activated chloride currents even though the cell became contracted in the presence of 1 uM Ca^{2+}. From these results, we could conclude that the increase of $[Ca^{2+}]_i$ to activate the outward Ca^{2+}-activated chloride current was mainly induced by the activation of the reverse mode of Na^+-Ca^{2+} exchanger. But for the increase of $[Ca^{2+}]_i$ to activate the inward tail current, L-type Ca^{2+} current may be the major provoking current. Since the cytosolic increase of $[Ca^{2+}]_i$ through pipette solution have failed to activate Ca^{2+}-activated chloride current, this chloride current may have very low Ca^{2+} sensitivity or a comparmental increase Ca^{2+} such as in subsarcolemmal space may activate the chloride current. Since there are several reports and models that the increase of Ca^{2+} in subsarcolemmal space would be over several to tens of uM, both possibility may be valid together.

Acknowledgement : This work was supported by grant No. (R01-2000-000-00172-0) from the Korea Science & Engineering Foundation and by the grant (No. IMT2000-C3-3) from the Ministry of Information and Communication.

References

Won Tae Kim, Han Choe, Yeon Jin Jang, Chun Sik Park, Chae Hun Leem, KJP, 6:S30, O3, 2002.