010P PHARMACOLOGICAL EVIDENCE FOR A KEY ROLE OF VOLTAGE-GATED K^+ CHANNELS IN THE FUNCTION OF RAT AORTIC SMOOTH MUSCLE CELLS

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Potassium (K^+) channels play an important role in the regulation of excitation-contraction coupling in vascular smooth muscle cells (VSMCs). Two major types of K^+ channel currents, large conductance Ca^{2+}-activated (BK_{Ca}) and voltage-dependent (I_{Kv}), are ubiquitously expressed in VSMCs. While I_{Kv} is mainly activated by membrane depolarisation, BK_{Ca} currents are also stimulated by an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) occurring in the presence of vasoconstrictors. The main aim of this study was to investigate a relative contribution of I_{Kv} and BK_{Ca} to the whole cell currents in rat aortic myocytes (RAMs) under increased $[Ca^{2+}]_i$ and to phenylephrine-induced contraction of rat aortic rings using selective K^+ channel pharmacology.

Thoracic aortas were isolated from male Wistar rats (225-300 g) humanely killed. Isometric tension recordings were performed from endothelium-denuded aortic rings (~ 3 mm in length) bathed in Krebs solution of the following composition (mM): 118 NaCl, 25 $NaHCO_3$, 4.9 KCl, 1.2 KH_2SO_4, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 11.7 glucose. Single RAMs were isolated using papain and collagenase (1 mg/ml each). K^+ currents were recorded with patch clamp technique at room temperature. Pipette solution contained (mM): 110 KCl, 10 NaCl, 5 $MgCl_2$, 10 HEPES, 10 EGTA and 5 mM $CaCl_2$ (calculated free $[Ca^{2+}]$=200 nM)and 7 mM $CaCl_2$ was used to achieve 444 nM $[Ca^{2+}]$. Perforated-patch recordings were performed using 100 μg/ml amphotericin B added to the pipette solution.

BK_{Ca} and I_{Kv} in the whole cell current were separated using 1 μM paxilline, a selective BK_{Ca} inhibitor. Electrophysiological analysis revealed that I_{Kv} activated at ≥-40 mV, while BK_{Ca} was seen positive to -20 mV in all three conditions. Voltage-dependent characteristics, but not maximal conductance, of I_{Kv} was significantly altered in increased $[Ca^{2+}]_i$. The most significant differences were observed in the I_{Kv} steady-state activation in $[Ca^{2+}]_i$ =444 nM (a significant shift by ~8 mV, $<0.011P<0.037$, unpaired *t* test). Cell dialysis with elevated $[Ca^{2+}]_i$ also shifted the I_{Kv} availability by ~10 mV ($0.008<P<0.012$, compared to perforate patch recordings). 1 μM correolide (a $K_V\alpha1$ blocker) did not inhibit the I_{Kv}. However, the I_{Kv} was blocked by tetra ethyl ammonium TEA (IC_{50}=3.1±0.6 mM, n=5) and by millimolar concentrations of 4-aminopyridine (4-AP). In non-stimulated aortic rings 1-5 mM TEA and 4-AP (inhibitors of I_{Kv}), but not paxilline (1 μM), caused contraction. Phenylephrine (15-40 nM) induced sustained tension with superimposed slow oscillatory waves (OWs) of contraction. OWs were blocked by diltiazem and ryanodine, suggesting the involvement of L-type Ca^{2+} channels and ryanodine-sensitive Ca^{2+} stores in this process. TEA and 4-AP (which block I_{Kv} in RAMs), but not IbTX and paxilline (BK_{Ca} inhibitors) nor correolide, increased the duration and amplitude of OWs. Our findings suggest that I_{Kv}, and not BK_{Ca}, plays an important role in the regulation of excitability of the rat aorta.