012P THE EFFECT OF ORGAN CULTURE AND ENDOTHELIN-1 TREATMENT ON Kv CURRENTS IN RAT SMALL PULMONARY ARTERIAL MYOCYTES

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In chronic hypoxia a decrease in the mRNA expression of Kv channels has been observed (Platoshyn *et al.,* 2001). Also, up-regulation of vasoconstrictors, endothelin-1 (ET-1) and thromboxane A_2, is evident (DiCarlo *et al.*, 1995), which may affect ion channel function. To enable the study of long-term effects of vasoactive agents on the function and expression of ion channels in blood vessels, we applied an organ culture technique, which has been used previously in functional studies (Murata *et al.*, 2001), to rat small pulmonary arteries (PAs).

Male Wistar rats (225-300g) were humanely killed, the heart and lungs removed *en bloc* and washed in sterile phosphate buffered saline. Small pulmonary arteries (400 μM external diameter) were micro-dissected, freed of connective tissue and incubated for 20-22 hours in DMEM 1% gentamycin in the absence or presence of 20 nM ET-1. No fetal calf serum (FCS) was used. Single PA myocytes (PAMs) were then isolated with papain (0.3 mg/ml) and collagenase (0.85 mg/ml) treatment for 20 min at 37º. Kv currents (I_{Kv}) were recorded using both perforated patch and whole cell patch-clamp techniques. Pipette solution (mM): 130 KCl, 0.5 $MgCl_2$, 0.5 $CaCl_2$, 10 HEPES, 10 EGTA, pH=7.2. For perforated patch 100μg/ml amphotericin B was added. I_{Kv} was isolated using 1 μM paxilline and 10 μM glibenclamide (to eliminate BK_{Ca} and K_{ATP} respectively) in the external solution containing (mM): 130 NaCl, 5 KCl, 1.2 $MgCl_2$, 1.5 $CaCl_2$, 10 HEPES, 10 glucose, pH=7.2.

Comparison of $I_{Kv,}$ measured with the perforated patch method, in PAMs isolated from fresh and organ-cultured PAs showed no significant difference in the current density at +100 mV (188±50 pA/pF, n=9 and 151±38, n=19, respectively, mean±s.e.m.), steady state activation or kinetics, indicating that organ culture does not affect I_{Kv}. An incubation of PAs in organ culture for 20-22 hours in the presence of 20 nM ET-1 resulted in significant changes in the voltage-dependent characteristics of I_{Kv}. The steady-state activation revealed a rightward shift with half-activation potential equal to 16.9±2.3 mV (n=17, control) and 25.1±3.7 (n=14, ET-1-treated, $P<0.033$, unpaired one tail *t* test). Also, a significant leftward shift in the I_{Kv} availability and decrease in the non-inactivating component of I_{Kv} were evident after ET-1 treatment. Acute application of 50 nM ET-1 on native cells however caused only a transient decrease in I_{Kv} amplitude.

In conclusion, we have shown that, 1) culturing of isolated small PAs in the absence of FCS for 22 hours does not significantly alter the properties of I_{Kv}; 2) a long-term ET-1 treatment significantly altered the properties of I_{Kv} via a yet to be identified mechanism. Therefore, organ culture can be successfully used to study the prolonged effects of vasoactive agents on the function of ion channels in blood vessels.

DiCarlo S. *et al*. (1995). *Am. J. Physiol.,* **269**, L690-697.

Murata T. *et al.* (2001). *Eur. J. Pharmacol.,* **421**, 45-53.

Platoshyn O. *et al.* (2001). *Am. J. Physiol.,* **280**, L801-812.