EFFECTS OF H_2O_2 ON Kv CURRENTS IN RAT PULMONARY ARTERIAL MYOCYTES (PAMS): THE ROLE OF INTRACELLULAR REDOX STATE

AL Smith, KH Yuill & SV Smirnov. Dept. Pharmacy & Pharmacology, University of Bath, Bath BA2 7AY, UK.

It has been proposed that changes in the intracellular redox state in PAMs during hypoxia could alter voltage-dependent K^+ (Kv) channel function. Hydrogen peroxide (H_2O_2), one of the main intracellular oxidants, was implicated in this process (Archer, 2002; Schumacker, 2001). However, the effect of H_2O_2 on Kv currents (I_{Kv}) in PAMs has not yet been characterised. Therefore, our main aim was to investigate the effect of 300 µM H_2O_2 on I_{Kv} using the conventional patch clamp technique at room temperature. Such a high concentration of H_2O_2 was chosen to maximise possible effects. Male Wistar rats (225-300 g) were humanely killed. PAMs were isolated from small pulmonary arteries (<400 µm external diameter) using 1 mg/ml collagenase and 0.5 mg/ml papain. The external solution contained (mM): 130 NaCl, 5 KCl, 1.2 $MgCl_2$, 1.5 $CaCl_2$, 10 HEPES, 10 glucose. 1 µM paxilline and 10 µM glibenclamide were also added to eliminate Ca^{2+}-activated and ATP-sensitive K^+ currents, respectively and PAMs were dialysed with (mM): 140 KCl, 0.5 $MgCl_2$, 0.5 $CaCl_2$, 10 HEPES, 10 EGTA, pH=7.2. For perforated patch recordings (PP) pipette solution contained: 140 KCl, 10 HEPES, 1 EGTA, 100 µg/ml Amphotericin B. Relative shifts in the half-activation and half-inactivation potentials (ΔV_a and ΔV_h, obtained from the Boltzmann fit of the I_{Kv} steady-state activation and availability respectively) were compared in the absence and presence of inhibitors in the same cell (Smirnov *et al*, 2002). All recordings were started 5 min after achieving the whole cell mode to allow adequate cell dialysis; PAMs were incubated for 5 min in H_2O_2 prior to any measurements. Data are expressed as mean±s.e.m. and compared using the students paired *t*-test ($p<0.05$ considered significant).

In PP 300 µM H_2O_2 had no effects upon activation, availability nor membrane potential. However, in the whole cell mode, it consistently reduced the whole cell I_{Kv} maximal conductance (G_{max}) by 15±4% (n=28, p<0.0001). At the same time H_2O_2 significantly shifted the I_{Kv} steady-state activation to more negative potentials (ΔV_a=-9±2.4 mV, n=28, p<0.001) without significant effect on I_{Kv} availability (ΔV_h=-3.4±2.1 mV, n=7, p>0.05). This effect was associated with a small (3.1±2.5mV, n=4) membrane hyperpolarisation induced by H_2O_2 in the current clamp mode, indicating overall stimulatory effect of H_2O_2 on the I_{Kv} in dialysed PAMs. Cell dialysis with 1 mM reduced glutathione (GSH, one of the main intracellular antioxidants) totally blocked the H_2O_2-induced shift in I_{Kv} activation (ΔV_a =2.8±3.1 mV, n=6, p>0.05). No significant effect on G_{max} was also observed under this condition.

Our findings, although being consistent with a stimulatory effect of H_2O_2 on I_{Kv} in PAMs described previously (Archer, 2002), nevertheless suggest that endogenous concentrations of intracellular antioxidants are sufficient to successfully neutralise the effect of even saturating amounts of H_2O_2 in intact rat small PAMs.

Archer SL *et al.* (2002). *Circ. Res*. **88**:1259-1266.
Schumacker PT *et al*. (2001). *Circ. Res.* **90**:1307-1915.
Smirnov SV *et al*. (2002). *J. Physiol.* **538**:867-878.