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Phenylephrine potentiates the relaxation to elevated potassium in rat isolated mesenteric arteries

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Rat isolated mesenteric arteries do not reliably relax to an increase of extracellular concentration of potassium ($[K^+]_o$). Mechanistic explanations for this differ between laboratories. The concentration of phenylephrine (PE) to which the arteries are exposed has been proposed to explain this discrepancy (Dora et al. 2002). Male Wistar rats (200-250 g) were killed humanely and sections of a third order branch of the superior mesenteric artery mounted for isometric tension recording in a Mulvany-type myograph containing bicarbonate-buffered physiological salt solution at 37°C. We found the concentration of PE or the resultant degree of tension development had no effect on the relaxation of arteries to an increase of $[K^+]_o$. In addition, inhibition of BK_{Ca} by TEA (5 mM), charybdotoxin (100 nM) or iberiotoxin (100 nM) did not induce a relaxation when $[K^+]_o$ was increased from 5.9 to 13.8 mM. The hyperpolarization and relaxation to an increase of $[K^+]_o$ from 4.6 to 13.8 mM was enhanced by increasing the duration of exposure to PE. The effect of duration of exposure to the vasoconstricting agonist was less pronounced when contraction was stimulated by U46619, a thromboxane A2 agonist (PE vs U46619, n=11 P <0.01; Mann-Whitney U test). Moreover, the time dependence of the effect of PE was significantly attenuated ($n = 8$, $P < 0.05$) in the presence of diphenylboric acid 2-aminoethyl ester (2-APB; 75 μM), an IP_3 receptor inhibitor, and abolished by SKF96365 (10 μM), a store-operated Ca^{2+} channel (SOC) inhibitor ($n = 8$, $P < 0.01$). In contrast, 50 nM ryanodine, a concentration previously shown to induce the Ca^{2+} release from intracellular stores (Meissner 1986), significantly enhanced the relaxation to raised $[K^+]_o$ ($n = 10$; $P < 0.005$). We have previously shown that relaxation of PE-induced force of rat isolated mesenteric artery by raised $[K^+]_o$ is dependent upon extracellular sodium, being depressed by a reduction in extracellular sodium and enhanced by monensin, in the presence but not absence of extracellular sodium (Brochet *et al.* 2002; Brochet & Langton, 2003). In this study we show that blockade of BK_{Ca} channels does not result in relaxation to elevated $[K^+]_o$. Increasing time of exposure to PE increased the amplitude and duration of the relaxation to raised $[K^+]_o$. Contractions induced by U46619, a vasoconstrictor that does not release SR calcium, were significantly less susceptible to relaxation to raised $[K^+]_o$ than contractions induced by PE. Finally, activation of SOCs by store emptying, using low concentrations of ryanodine, augmented the $[K^+]_o$-induced relaxation of PE-induced contractions. We interpret these data to suggest that augmentation the accumulation of $[Na^+]_i$ following activation of SOCs by PE and the depression of Na,K-ATPase activity in low $[K^+]_o$ both serve to potentiate the upturn in the activity of the Na,K-ATPase when $[K^+]_o$ is subsequently increased. This results in hyperpolarization and relaxation of the arterial smooth muscle.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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The effect of mitochondrial electron transport chain (ETC) inhibitors on voltage-dependent K^+ (Kv) currents in rat small pulmonary arterial myocytes (PAMs)

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Evidence suggests that Kv channel activity in PAMs may be altered by inhibition of mitochondrial function. Therefore, the effect of ETC inhibitors myxothiazol, antimycin A (AA) and rotenone (all 1 μM), on Kv currents (I_{Kv}) was investigated using a patch clamp technique at room temperature. Male Wistar rats (225-300 g) were humanely killed. PAMs were isolated from small pulmonary arteries (⟨400 μM external diameter) using collagenase (1 mg/ml) and papain (0.5 mg/ml). I_{Kv} was isolated using 1 μM paxilline and 10 μM glibenclamide to eliminate Ca^{2+} activated and ATP-sensitive K^+ currents, respectively. The external solution contained (mM): 130 NaCl, 5 KCl, 1.2 $MgCl_2$, 1.5 $CaCl_2$, 10 HEPES, 10 glucose and PAMs were dialysed with (mM): 140 KCl, 0.5 $MgCl_2$, 0.5 $CaCl_2$, 10 HEPES, 10 EGTA, pH=7.2. Effects on I_{Kv} were compared in terms of relative shifts in the half-activation, V_a, and half-inactivation, V_h, potentials (ΔV_a and ΔV_h, respectively, obtained in the absence and presence of inhibitors in the same PAM) derived from the Boltzmann fit of the steady-state activation and availability of I_{Kv}. Holding potential was -80 mV. Data are expressed as mean±s.e.m. and compared using the students paired *t*-test (p⟨0.05 considered significant).

Rotenone (a complex I inhibitor) had no effect upon I_{Kv} activation but significantly shifted the I_{Kv} availability to more negative voltages (ΔV_h=-5.1±1.9 mV, n=7, p⟨0.04). The slope factor (k_h) was also significantly reduced (8.6±1.1 mV vs. 11.8±1.2 mV, control, n=7, p⟨0.03). AA and myxothiazol inhibit ETC by blocking proximal and distal to ubisemiquinone in the enzyme Q cycle in complex III, respectively. Whilst myxothiazol had no effect upon ΔV_a or ΔV_h, AA caused a significant leftward shift in both I_{Kv} activation (ΔV_a=-15.4±3 mV) and inactivation (ΔV_h=-12±3.2 mV, n=7, p⟨0.01). Significant increases in the slope of activation and inactivation dependencies were also observed (0.01⟨p⟨0.04).

To study the involvement of cell redox state, the effect of hydrogen peroxide (H_2O_2) and reduced glutathione (GSH) on rotenone and AA was evaluated. Application of 300 μM H_2O_2 caused a leftward shift in I_{Kv} activation but not inactivation and pre-treatment with H_2O_2 did not significantly block effects of AA. Interestingly with rotenone, a significant rightward shift in I_{Kv} activation was observed in the presence of H_2O_2 (ΔV_a=6.1±1.9 mV, n=6, p⟨0.05). Cell dialysis with 1 mM GSH blocked rotenone-induced shift in I_{Kv} inactivation (V_h=-0.7±2.8 mV, n=7), however a significant shift in both activation (ΔV_a=-7.1±3 mV, n=15) and inactivation (ΔV_h=-8.7±2.5 mV, n=14) dependencies was seen with AA. Our findings suggest that the effects of AA, but

not rotenone, on I_{Kv} in PAMs appear to be independent of cell redox state.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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The mechanisms of nitric oxide relaxing action on the smooth muscles of the skeletal musculature vessels

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Title only.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.