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Effects of potassium channel inhibitors upon the electrophysiology of pericytes on descending vasa recta (DVR) isolated from rats

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DVR are vasoactive, consisting of endothelium surrounded at intervals by pericytes, and may control distribution of renal medullary blood flow (Zhang *et al.* 2001, 2002). Depolarisation apparently triggers contraction of these pericytes, as of many smooth muscle cells, by opening calcium channels. These pericytes are depolarised by chloride currents (activated by angiotensin II) or by extracellular potassium (Zhang *et al.* 2001, 2002), but their potassium channels are unknown. Smooth muscle may express calcium-activated (K_{Ca}), voltage-dependent (K_v) and inward rectifier (K_{IR}) potassium channels, inhibited by tetraethylammonium chloride (TEACl), 4-aminopyridine (4AP) and $BaCl_2$, respectively (Nelson & Quayle, 1995). TEACl (30 mM) and $BaCl_2$ (1 mM) depolarise pericytes on DVR (Zhang *et al.* 2002), but are nonspecific at such concentrations (Nelson & Quayle, 1995). This abstract describes the addition of TEACl (2 mM), 4AP (5 mM) and $BaCl_2$ (50 μM) during whole cell perforated patch clamp recording of currents from pericytes on isolated DVR.Individual DVR were dissected from renal tissue kept at 4 °C (Zhang *et al.* 2001, 2002), after removal from rats humanely killed by stunning and cervical dislocation. DVR were incubated in collagenase and hyaluronidase (0.4 mg ml^{-1} of each) at room temperature for 8-9 min, stored on ice and transferred at intervals to solution at room temperature, containing (mM) Na^+ 150, K^+ 5, Mg^{2+} 1, Ca^{2+} 1, Cl^- 159, HEPES 10 and glucose 10, plus 18β-glycyrrhetinic acid (40 μM), a gap junction blocker (Turner, 2003). Heat polished pipettes containing a solution of Na^+ 10, K^+ 140, Cl^- 150 and HEPES 10, plus gramicidin (0.4 mg ml^{-1}) and dimethylsulfoxide (0.4%), were applied to pericytes to form gigaohm seals. Pericytes were clamped at -50 mV and exposed every 5 s to a ramp from -140 to +50 mV (1 mV ms^{-1}). TEACl was added to pericytes in control solution, 4AP to pericytes in TEACl and $BaCl_2$ to pericytes in TEACl plus 4AP. These inhibitors did not alter currents significantly (Fig 1) and therefore provide no evidence for K_{Ca}, K_v or K_{IR} currents in unstimulated pericytes on DVR.

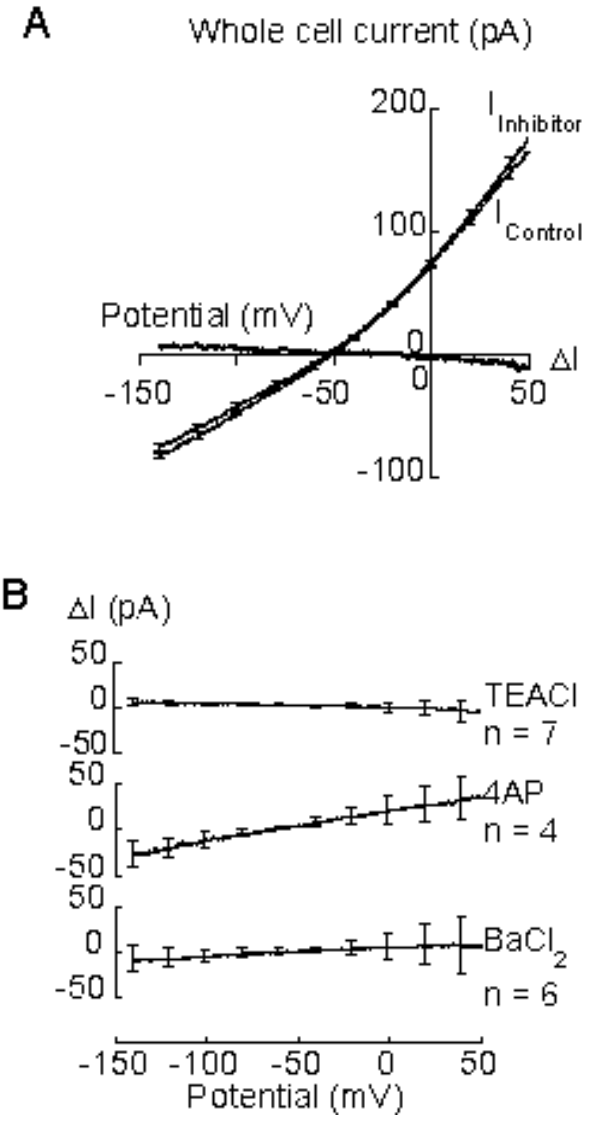

Figure 1. A. Mean pericyte current (from 4 successive ramps) just before ($I_{Control}$) and 1 min after ($I_{Inhibitor}$) addition of TEACl. Most standard error bars omitted, for clarity. $\triangle I = I_{Control} - I_{Inhibitor}$. B. $\triangle I$ calculated from currents just before and 0.5 - 3.3 min after addition of TEACl, 4AP or $BaCl_2$. $\triangle I$ is not significantly different from zero (P>0.10, unpaired t test).

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

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Inhibition of Protease-Activated Receptor-2 (PAR-2) Attenuates Joint Inflammation

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Protease-activated receptors are activated by protease cleavage of the N terminus, generating a tethered ligand that interacts with extracellular loop-2. Protease-activated receptor-2 (PAR-2) is a member of this family involved in inflammatory processes as adjuvant-induced arthritis is substantially attenuated in PAR-2-deficient mice compared to wild-type mice (Ferrell et al. 2003). Here we investigate PAR-2 as a therapeutic target in arthritis using a polyclonal antibody (B5) to the trypsin-binding site on PAR-2 in a model of acute arthritis. The specificity of B5 was confirmed in parallel groups, joint swelling being induced by a synthetic PAR-2 agonist or the PAR-1 activating peptide, thrombin. Acute knee joint inflammation was induced by intra-articular (i.a.) injection of a 2% carrageenan and 4% kaolin (CK; 20ml) in deeply anaesthetised (O_2/N_2O/2% halothane mixture, withdrawal reflex absent) male C57BL/6J mice (n=6). In a parallel group (n=6), B5 (20ml; 1:1000 dilution) was administered by i.a. injection 5 minutes prior to CK. A further group (n=6) was pre-treated with non-immune serum. Swelling at 24 hours was expressed as % of pre-injection values. In a separate group, swelling was induced by i.a. injection (100 mg) of a synthetic PAR-2 agonist (Ferrell et al 2003), or thrombin (20 Units). In parallel groups (n=4/group) B5 was administered and joint diameter measured as above. All animals were humanely killed. Data expressed as mean ± S.E.M. and analysed by Students unpaired t test.CK alone resulted in a 26 ± 4% increase in joint diameter at 24 hours that was significantly attenuated ($P < 0.002$) by B5 (6 ± 2%). The CK alone response was not significantly (P=0.36) altered by non-immune serum (20 ± 4%). The PAR-2 agonist increased joint diameter (17 ± 3%) which was significantly attenuated (P=0.01) by B5 (4 ± 1%). Thrombin produced a smaller increase in diameter (7 ± 2%) that was not significantly reduced by B5 (P=0.55), arguing that the B5 effect is specific for PAR-2. The fourfold reduction of CK-induced joint swelling suggests that inhibition of PAR-2 activation is anti-inflammatory. B5 is specific for PAR-2 and its inhibition of the PAR-2 agonist argues that this antibody attenuates acute joint inflammation by receptor blockade rather than by preventing generation of the tethered ligand. These results, using a pharmacological tool, confirms and extends our earlier findings, supporting the concept of PAR-2 as therapeutic target for future studies in arthritis.

Ferrell, WR *et. al.* (2003) *J Clin Invest* **111**, 34-41.

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