Effects of AT_1 and AT_2 receptor antagonists upon whole cell currents in pericytes on descending vasa recta isolated from rat kidneys and exposed to angiotensin II

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Angiotensin II (AngII) constricts isolated descending vasa recta (DVR), apparently by contracting surrounding pericytes, at least partly by stimulating chloride currents (Turner *et al.* 2001; Zhang *et al.* 2001; Pallone & Huang, 2002). Such vasoactivity suggests that DVR may regulate the distribution of blood flow within the renal medulla. Blocking AngII receptor subtypes indicates that AT_1 stimulation constricts DVR, whereas AT_2 stimulation opposes constriction (T.L. Pallone, personal communication). I have looked for effects of selective AT_1 or AT_2 antagonists, losartan and PD123,319, respectively, upon pericyte currents stimulated by AngII, during whole-cell permeabilised patch-clamp recording from DVR.

Individual DVR were dissected from renal tissue kept at 4 °C (Zhang *et al.* 2001), 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 at 4 °C 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 (Yamamoto *et al.* 1998). Heat-polished pipettes filled with solution containing Na^+ 10, K^+ 140, Cl^- 150 and Hepes 10, plus gramicidin (0.4 mg ml^{-1}), were applied to pericyte cell bodies to form gigaohm seals and AngII was added once to each microvessel.

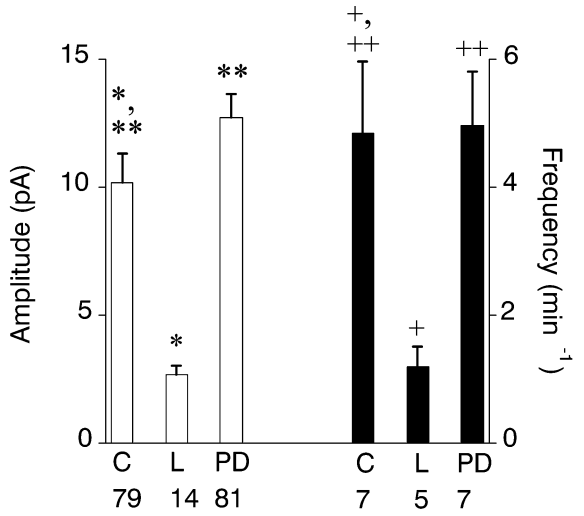

Figure 1. Amplitude (□) and frequency (■) of pericyte transient currents during exposure to AngII, either alone (controls, C) or after addition of losartan (L) or PD123,319 (PD). Means ± S.E.M. *$P < 0.0001$, **$P > 0.05$, +$P < 0.05$, ++$P > 0.10$ (Student's unpaired t test). Numbers of transients (amplitude) or pericytes (frequency) are shown below the abscissa.

AngII stimulated repetitive transient inward currents in pericytes at −50 mV (Turner *et al.* 2001; Pallone & Huang, 2002). Losartan (10^{-6} M) reduced the amplitude and frequency of transients between 40 and 180 s of exposure to AngII (10^{-8} M), but PD123,319 (10^{-6} M) did not modify them significantly (Fig. 1). This is evidence that AngII acts via AT_1, but not AT_2, receptors to modulate these currents.

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All procedures accord with current UK legislation.

Hypoxia-induced dilatation of rat coronary resistance arteries: effects of glibenclamide

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Autoregulation and metabolic dilatation are major determinants of tone in the coronary circulation. Pressure-dependent myogenic tone may be important for autoregulation and K_{ATP} channels may be involved in metabolic dilatation. We have previously reported that K_{ATP} channels may not be required for adenosine-mediated dilatation in rat or human coronary resistance arteries (Lynch *et al.* 2002). The effect of decreased O_2 on resistance arteries has not been directly studied. The aims of the current study were to demonstrate a hypoxic response in rat coronary resistance arteries with myogenic tone and to determine whether K_{ATP} channels are involved this response.

Wistar rats were killed by cervical dislocation and septal coronary arteries were dissected. Arteries ($n = 7$) were pressurised to 60 mmHg and checked for leaks. The inner diameter and wall thickness was continually monitored using a video dimension analyser. Once myogenic tone stabilized, the myogenic reactivity of vessels was determined by reducing pressure to 20 mmHg and then increasing it in 20 mmHg increments to 100 mmHg. Vessels were returned to 60 mmHg before undergoing a 10 min hypoxic challenge (I) after which normoxic (95 % air and 5 % CO_2) conditions were restored. Hypoxia (< 10 mmHg O_2) was induced by switching to a 95 % N_2 and 5 % CO_2 gas mixture. When tone returned to pre-hypoxic levels, vessels were subjected to a second hypoxic challenge (II). After 10 min glibenclamide (5×10^{-6} M) was added to the bath. Hypoxia caused a dilatation of the rat coronary artery with myogenic tone. This was unaffected by glibenclamide. Mean lumen diameters (μm) (± S.E.M.) are shown in Table 1.

Table 1. Mean lumen diameters (μm)

Challenge	I	II
Pre-hypoxia	151.6 (8.3)	148.6 (8.9)
Hypoxia	181.5 (10.2)*	170.4 (8.3)*
Plus glibenclamide	—	171.0 (9.2)
Post-hypoxia	149.4 (9.2)†	149.0 (7.3)†

*Significantly different from pre-hypoxia ($P < 0.05$, paired t test).
†Significantly different from hypoxia ($P < 0.05$, paired t test).

It has been shown that replacement of glucose with 2-deoxyglucose can inhibit glycolysis and glycogenesis, and under these conditions rat coronary arteries can produce a

hypoxic-like dilatation (Conway *et al.* 1994). In our preparation ($n = 6$) 2-deoxyglucose significantly ($P < 0.05$, paired t test) dilated a vessel with myogenic tone, from a diameter of 176 (11) μm to a diameter of 215 (2.7) μm. The dilatation persisted in the presence of glibenclamide (209.0 (13.6) μm).

This study implies that K_{ATP} channels are not required for hypoxic-induced relaxation of coronary resistance arteries in the rat. K_{ATP} channels do not appear to play a role in the dilator response to 2-deoxyglucose in rat coronary arteries with myogenic tone.

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