

## PS C12

**Comparison of endothelial-dependent relaxations of isolated mouse mesenteric and human myometrial resistance arteries**

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Preeclampsia (PE) is a pregnancy-specific syndrome attributable to endothelial dysfunction. This dysfunction has been induced in isolated human myometrial arteries by plasma obtained from women with PE, pre- and post-diagnosis (Hayman *et al.* 2000, Myers *et al.* 2003). Characterisation of vasoactive factors within such plasma promises to increase our understanding of the pathophysiology of PE. We have studied human myometrial vessels by small vessel wire myography, but patient and vessel variability is marked and sample availability limited. Mouse mesenteric vessels may represent a more appropriate stringent model to characterise these plasma factors.

Ethical approval was obtained for experiments on human tissue. With written informed consent myometrial samples were taken at Caesarean section from women ( $n = 10$ ) with uncomplicated pregnancies at term. Male C57B mice ( $n = 8$ ) were humanely killed by stunning, followed by cervical dislocation. Myometrial or mesenteric vessels were mounted on a wire myograph and normalised in physiological salt solution (95% air–5% CO<sub>2</sub>; 37°C). Arteries were constricted to incremental doses of arginine vasopressin (AVP) ( $10^{-10}$ – $10^{-8}$  M), U46619 ( $10^{-10}$ – $10^{-6}$  M) or phenylephrine (Phe) ( $10^{-9}$ – $10^{-5}$  M), and exposed to the endothelial-dependent vasodilators bradykinin (BK) ( $10^{-10}$ – $10^{-6}$  M) or acetylcholine (ACh) ( $10^{-9}$ – $10^{-5}$  M). Mechanisms of endothelial-dependent relaxation were elucidated by prostacyclin and NO blockade with indomethacin (10 µM) and *N*-nitro-L-arginine (LNNA; 100 µM), respectively.

Mouse mesenteric and human myometrial vessels constricted to all agonists. In mouse mesenteric arteries, contractile responses to AVP were oscillatory but sustained to Phe or U46619. In contrast, sustained constrictions were produced by human myometrial vessels in response to AVP and U46619, but not to Phe. Mesenteric vessels relaxed in a dose-dependent manner to ACh, but not to BK, whereas myometrial vessels relaxed to BK, but not to ACh. Coincubation with indomethacin and LNNA, attenuated maximal ACh-induced relaxation vs. control vessels (maximal relaxation  $56.2 \pm 9.61\%$  vs.  $23.1 \pm 4.3\%$ ; means  $\pm$  S.E.M.; Student's unpaired *t* test,  $P \leq 0.05$ ). The ACh-dependent NO/prostacyclin-independent relaxation of mouse mesenteric vessels was similar to the BK-dependent NO/prostacyclin-independent relaxation of human myometrial vessels (maximal relaxation  $55.4 \pm 12.6\%$  vs.  $14.1 \pm 5.4\%$ ;  $P \leq 0.05$ ).

Thus, mouse mesenteric and human myometrial vessels differed in agonist-mediated endothelial-dependent relaxations. However, the NO/prostacyclin-independent component of agonist-induced vasodilatation, attributable to EDHF, was similar in both vascular beds.

Hayman RH *et al.* (2000). *BJOG*, 108–15.Myers JE *et al.* (2003). *J Soc Gynecol Invest* 10, 172A.

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All procedures accord with UK legislation, local guidelines and the Declaration of Helsinki

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## PS P41

**Effect of long-term, severe hypoxia on vascular tone of isolated, pressurised rat mesenteric small arteries**

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We have previously shown that acute (5 min) periods of severe hypoxia cause partial dilatation of depolarised or agonist-contracted pressurised rat mesenteric small arteries (Shaw *et al.* 2003). This study examines the effect of extended periods of severe hypoxia on contractions elicited to various stimuli in pressurised rat mesenteric arteries.

Small mesenteric arteries (100–200  $\mu\text{m}$  internal diameter) were isolated from male Wistar rats (humanely killed by cervical dislocation following stunning), cannulated and mounted on a pressure myograph. Vessels were pressurised to 60 mmHg and perfused with bicarbonate-buffered saline at 37°C, gassed with 95 % air–5 %  $\text{CO}_2$  and left to equilibrate for 30 min. The  $P_{\text{O}_2}$  of the superfusate was continuously measured by placing a small  $\text{O}_2$  probe (Instech Laboratories) 1–2 mm from the artery ( $P_{\text{O}_2} = 135.4 \pm 3.3$  mmHg (mean  $\pm$  S.E.M.),  $n = 12$  animals). Intraluminal diameter was continuously monitored. Vessels were stimulated with high  $\text{K}^+$  solution (60 mM KCl isosmotically substituted for NaCl), 10  $\mu\text{M}$  phenylephrine (PE) or 10  $\mu\text{M}$  U46619. Severe hypoxia was achieved by gassing with 95 %  $\text{N}_2$ –5 %  $\text{CO}_2$  and determined when superfusate  $P_{\text{O}_2} < 10$  mmHg (time taken =  $7.2 \pm 0.9$  min,  $n = 12$ ).

Resting diameter of arteries in normoxia was  $178.3 \pm 11.6$   $\mu\text{m}$  ( $n = 12$ ). Addition of high  $\text{K}^+$  solution, PE or U46619 produced a rapid constriction of similar magnitude in all vessels (changes in diameter were  $147.5 \pm 16.7$   $\mu\text{m}$  ( $n = 6$ ),  $121.7 \pm 10.9$   $\mu\text{m}$  ( $n = 3$ ) and  $93.3 \pm 17.6$   $\mu\text{m}$  ( $n = 3$ ) for high  $\text{K}^+$ , PE and U46619, respectively). Severe hypoxia dilated PE pre-constricted arteries by  $97.5 \pm 1.3$  % (time for maximum response =  $19.3 \pm 4.5$  min). Significantly smaller dilatory responses were observed in tissues contracted by high  $\text{K}^+$  (maximum dilatation of  $67.6 \pm 3.5$  %, time =  $21.8 \pm 3.7$  min) or by U46619 (maximum dilatation  $66.4 \pm 11.0$  %, time =  $82.0 \pm 19.6$  min) ( $P < 0.05$ , Student's unpaired  $t$  test). Following maximal dilatation, depolarised arteries re-constricted after 2 h in the continued presence of hypoxia to  $96.7 \pm 8.3$  % of the high  $\text{K}^+$  constricted tone in normoxia. However, arteries pre-constricted by either PE or U46619 and subsequently dilated with hypoxia did not re-constrict in the continued presence of lowered oxygenation. Thus, dilatations to long-term, severe hypoxia were greater in PE compared to high  $\text{K}^+$  or U46619 pre-constricted pressurised rat mesenteric arteries. Vessels pre-constricted by high  $\text{K}^+$ , but not by PE or U46619, exhibited a biphasic response to lowered oxygen and, with time, re-constricted fully in the continued presence of hypoxia.

Shaw L *et al.* (2003). *J Physiol* (in press).

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All procedures accord with current UK legislation

## PS P42

**Protective effects of a medicinal plant extract against D-glucose-induced cytotoxicity in cultured bovine retinal pericytes**

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Diabetic retinopathy is one of the microvascular complications in diabetes. The hallmark of early diabetic retinopathy is the loss of pericytes (Cogan *et al.* 1961). These changes lead to decreased capillary tonicity, increased retinal blood flow, formation of microaneurysms and uncontrolled proliferation of endothelial cells. Both the Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) have shown that hyperglycaemia is a major factor in the onset and progression of microvascular complications in diabetic patients (DCCT Research Group, 1993; UKPDS Group, 1998). The aim of this study is to evaluate the therapeutic potential of *Gynura procumbens* to prevent diabetic retinopathy. *G. procumbens*, a vigorous climbing herb belonging to the family Compositae, is found in various parts of Southeast Asia. The *G. procumbens* leaf is edible and has been reported to have antihyperglycaemic activity (Zhang & Tan, 2000). The methanol extract of *G. procumbens* leaf was evaluated for protection of cultured bovine retinal pericytes (BRP) against D-glucose-induced toxicity.

Subconfluence BRP (from eyes obtained from an abattoir) were incubated in 5 mM or 25 mM D-glucose, with or without the extract for 4 days. The protective effect of the extract on D-glucose-induced toxicity was determined by using Trypan Blue staining. Further, intracellular glucose concentrations were measured using the Amplex Red kit (Molecular Probes) and expression of GLUT1 protein determined by immunoblotting.

Incubation of BRP in 25 mM D-glucose for 4 days reduced the number of viable cells to  $75 \pm 2$  % (mean  $\pm$  S.E.M.,  $n = 12$ ) compared to the BRP in 5 mM D-glucose. The addition of non-toxic concentrations ( $2$ – $10$   $\mu\text{g ml}^{-1}$ ) of methanol leaf extract of *G. procumbens* significantly reversed the adverse effects of elevated D-glucose. Chronic exposure of the BRP to high glucose led to an increased accumulation of intracellular glucose ( $282 \pm 52$  vs.  $98 \pm 16$  nmol (mg protein) $^{-1}$  in 5 mM glucose, means  $\pm$  S.E.M.,  $n = 3$ , Student's unpaired  $t$  test,  $P < 0.01$ ) and downregulation of GLUT1 protein (20 % vs. 5 mM glucose). These effects were partially reversed by *G. procumbens* at a dose of  $10$   $\mu\text{g ml}^{-1}$ .

In conclusion, *G. procumbens* may be of therapeutic potential for the treatment of diabetic retinopathy.

Cogan D *et al.* (1961). *Arch Ophthalmol* **66**, 366–378.

DCCT Research Group (1993). *New Eng J Med* **329**, 977–986.

UKPDS Group (1998). *Lancet* **352**, 837–853.

Zhang XF & Tan BK (2000). *Singapore Med J* **41**, 9–13.

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