C119

Adrenomedullin and CGRP interact with endogenous calcitonin-receptor-like receptor in human endothelial cells and induce its desensitisation by two distinct mechanisms

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Adrenomedullin (AM) and calcitonin gene-related peptide (CGRP) are related peptides with distinct pharmacological profiles. Calcitonin-receptor-like receptor (CRLR, now known as CL) can function as either an AM receptor or a CGRP receptor, when cotransfected with receptor-activity-modifying proteins (RAMPs) that define ligand-binding specificity (McLatchie et al. 1998). The aim of the present study was to determine the role of endogenously expressed CL (EndoCL) in generating endogenous AM and CGRP receptors. We raised anti-human CL antibody and identified microvascular endothelial cells (MVECs) as a major CL-expressing cell type in tissues by immunohistochemistry. Cultured MVECs continue to express EndoCL as well as fully active endogenous AM- and CGRP-sensitive receptors in vitro, as demonstrated by the ability of both peptides to induce dose-dependent (10nM to 1μM) migration and cAMP accumulation ($P<0.05$), as well as Akt phosphorylation. We therefore tested the hypothesis that endothelial EndoCL can interact with both AM and CGRP by examining receptor internalisation (accessed by immunofluorescence in three independent experiments) and desensitisation (loss of the ability to induce Akt phosphorylation). We found that agonist-mediated internalisation of EndoCL occurs in response to AM but not CGRP (10nM to 1μM) in MVECs. However, AM-induced (100 nM) EndoCL internalisation was blocked by antagonists of both AM and CGRP receptors: AM(22-52) and CGRP(8-37) (1μM), respectively. Furthermore, AM-induced EndoCL internalisation resulted in desensitisation not only of AM but also of CGRP receptors. Finally, CGRP (100 nM) also induced desensitisation of both endogenous AM and CGRP receptors, but did not mediate EndoCL internalisation despite interaction with this receptor. Thus, EndoCL interacts with both AM and CGRP, and simultaneously acts as a receptor for both peptides (i.e. acting as an endogenous AM/CGRP receptor) in endothelial cells. Interaction with either ligand is sufficient to induce EndoCL desensitisation to both AM and CGRP, but differential mechanisms are involved since only AM induces EndoCL internalisation. These novel findings regarding regulation of EndoCL function in endothelial cells are likely to be of importance in conditions where AM or CGRP levels are elevated, such as cardiovascular disease, diabetes and inflammation (Nikitenko et al. 2006).

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

C120

Raised plasma glucose attenuates the acute permeability response to glycated albumin in the isolated perfused retina

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Raised vascular permeability precedes the clinical appearance of diabetic retinopathy and is closely correlated with the degree of hyperglycaemia and formation and accumulation of advanced glycation end products (AGEs) in the retinal vasculature. We have previously shown, using the isolated perfused retina, that retinal capillary permeability increased rapidly (< 30 s) in response to abluminal application of AGE-BSA via activation of the receptor for AGE (RAGE) and production of reactive oxygen species (ROS) from NADPH oxidase (Warboys & Fraser, 2005). We now report on the interaction between increased concentrations of glucose and AGE-BSA and the effects on retinal microvascular permeability.

Retinae, obtained from Wistar rats, were mounted under a fluorescence microscope and continuously superfused with a Hepes buffer, pH 7.4, 37°C. The microvasculature was perfused with sulforhodamine dye via a micropipette inserted into a radial artery and permeability determined from the rate of decrease in fluorescence gradient across a venular capillary at zero flow and ambient pressure.

A 15 min abluminal application of 25 mM glucose resulted in an initial (< 30 s) increase in permeability from 0.07 ± 0.03 to $1.27 \pm 0.3 \times 10^{-6}$ cm.s^{-1} ($P < 0.01$, paired t test, mean ± sem) that rapidly returned to basal values. This response was unaffected by RAGE blockade with 50 μg.ml^{-1} anti-RAGE IgG (n = 4) or inhibition of NADPH oxidase with 100 μM apocynin (n = 10). No further increase in permeability was observed throughout the 15 min application, however, the response to AGE-BSA was significantly attenuated from 1.02 ± 0.1 to $0.31 \pm 0.1 \times 10^{-6}$ cm.s^{-1} ($P < 0.001$, n = 6). Similar treatments with osmotic controls were without effect. Furthermore, the response to AGE-BSA was also suppressed in ZDF rats, a model of Type 2 diabetes (blood glucose 21 ± 1.8 mM), compared to non-diabetic controls (blood glucose 5 ± 0.2 mM), with the maximal permeability response reduced from 1.34 ± 0.2 to $0.77 \pm 0.4 \times 10^{-6}$ cm.s^{-1} ($P < 0.05$, 2-way ANOVA, n = 3).

The acute response to bradykinin (Bk) which requires ROS production, independent of NADPH oxidase activation, was unaffected by pre-treatment with 25 mM glucose. On the other hand, a 10 minute pre-treatment with AGE-BSA resulted in a leftward shift and elevation of the Bk dose-response curve suggesting potentiation of the acute response to Bk. This potentiation was abolished following inhibition of NADPH oxidase with apocynin. These data suggest a complex interplay between signal transduction pathways that are dependent on the rapid formation of ROS.

Warboys CM & Fraser PA (2005). *J Physiol* **565P**, C133.

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C121

Acute activation of nitric oxide synthase by isoflavones in human vascular endothelial cells. Role of extracellular signal-regulated kinase, Akt and HSP90

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Oestrogen protects premenopausal women against heart disease; however, hormone replacement therapy following menopause has been linked to breast cancer and has limited benefits on the cardiovascular system (Rossouw *et al.* 2002). Dietary phytoestrogens, such as genistein and the isoflavone metabolite equol, may serve as alternative oestrogen receptor modulators that prevent vascular endothelial dysfunction through enhanced generation of the vasodilator nitric oxide (NO; Kuiper *et al.* 1998; Mahn *et al.* 2005). The present study investigates whether genistein or equol can acutely modulate intracellular signalling pathways involved in the activation of endothelial NO synthase (eNOS). Confluent cultures of human umbilical vein endothelial cells (HUVEC) were treated acutely (0-10 min) with either genistein or equol (100nM) in Krebs Henseleit buffer containing L-arginine (100 μM) and lysates collected for Western blot analyses. The statistical signigficance of quantified data derived from n=3-6 independent HUVEC cultures were evaluated using Student's unpaired t tests. Immunoblotting revealed that eNOS-Ser^{1177} was phosphorylated by equol at 2-10 min with concurrent phosphorylation of Akt and extracellular signal-regulated kinase (ERK1/2). Dissociation of caveolin-1 from eNOS and increased eNOS association with HSP90 following equol treatments was demonstrated by eNOS immunoprecipitation. Inhibition of phosphoinositol-3-kinase/Akt using LY294002 (10 μM, 30 min) or MEK1/2 using or U0126 (1 μM, 30 min) resulted in a significant attenuation of eNOS phosphorylation by equol (2 min, 100 nM, $p<0.05$, n=3). Our present study provides the first direct evidence that isoflavones can acutely phosphorylate eNOS in HUVEC via Akt and ERK1/2 activation to enhance eNOS dissociation from caveolin-1 and association with HSP90. These acute intracellular signalling events mediate the enhanced NO generation and vascular relaxation elicited by isoflavones.

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C122

In utero protein restriction leads to a down-regulation of antioxidant gene expression in adult male offspring

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In rats, restriction of dietary protein during pregnancy leads to raised blood pressure, impaired vasodilatation (Brawley et al. 2003) and increased oxidative damage (increased protein carbonyl concentration) (Langley-Evans et al. 2005) in the male offspring. The aim of the present study was to assess mRNA expression of three key endothelial antioxidant genes, heme oxygenase-1 (HO-1), glutamate cysteine ligase (GCL) and manganese superoxide dismutase (Mn-SOD), as well as, endothelial nitric oxide synthase (eNOS), in the liver and mesenteric arteries of adult male offspring of protein-restricted rat dams.

Pregnant Wistar rats (240 g) were fed either a control diet (C; 18% casein, n = 6) or a protein-restricted diet (PR; 9% casein, n = 6) throughout gestation from conception. At 120 days of age, male offspring were culled and liver and small mesenteric arteries (~250 μm diameter and ~12 arteries per animal) were removed and snap frozen in liquid nitrogen. RNA was extracted and mRNA levels of HO-1, GCL, MnSOD and eNOS were measured using quantitative real-time PCR and normalised with respect to 28S RNA (Mahn et al. 2005). Data are presented as mean ± SEM, and differences assessed by two-way analysis of variance (ANOVA) with significance accepted at $p<0.05$.

The mRNA expression of all three antioxidant genes was reduced in the livers of the PR compared to C male offspring (HO-1: C; 1807 ± 439, n = 11, PR; 424 ± 109, n = 11, p = 0.006, GCL: C; 120 ± 16, n = 11, PR; 70 ± 11, n = 11, p = 0.016, MnSOD: C; 1544 ± 189, n = 11, PR; 1044 ± 101, n = 12, p = 0.027, figures expressed as gene mRNA relative to 28s RNA). Expression in the mesenteric arteries followed the same trend, being reduced by 14% for HO-1, 36% for MnSOD and 36% for GCL but did not reach significance for any of the three genes. The eNOS mRNA expression was not significantly different between the two groups in either tissue type.

These findings demonstrate that the previously observed oxidative damage in the offspring of in utero protein-restricted rats (Langley-Evans et al. 2005) may be in part due to a reduced antioxidant enzyme defence. Diminished antioxidant defences may also account for the previously observed vascular dysfunction in the mesenteric arteries due to a nitric oxide–reactive oxygen species imbalance in the PR offspring.

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PC193

Single leg vein properties measured by ultrasound during the menstrual cycle and oral contraceptive use in women

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Studies of the effects of female hormones on venous function have reported increased distensibility during the luteal phase of the normal menstrual cycle and during oral contraceptive use (Fawer *et al.* 1978) or no difference (Meendering *et al.* 2004). These results are based on measurements of whole limb volume changes to venous occlusion. As this includes additional tissues it may not represent venous volume accurately.

Doppler ultrasound imaging (L12 – 3 probe, Philips UK) was used to investigate the effect of female hormones on the capacitance and compliance of the saphenous vein on the medial aspect of the calf of young women. Eight women (age 20 ± 2 (SD) yrs, height 166 ± 8 cm, weight 66 ± 16 kg), four not taking (NOC) and four taking (OC) oral contraceptives, were tested during the menstrual (M, days 2 – 7) and luteal (L, days 23 – 27) phases of the menstrual cycle. Subjects lay in a supine position with legs supported at heart level. Venous diameter was measured using on-line vessel border detection software (VIA Software, MD Medic) on a longitudinal image. Baseline values were taken after 10 min rest and showed no significant difference between groups (NOC M 2.84 ± 1.49 mm (mean ± S.E.M), NOC L 3.21 ± 1.99 mm, OC M 1.83 ± 0.79 mm, OC L 2.01 ± 0.34 mm, NS by repeated measures ANOVA). After 5 min venous distension (thigh cuff inflation to 60 mmHg) vein diameter increased similarly in NOC and OC women during both phases (NOC M 117.1 ± 5.4%, NOC L 116.4 ± 5.1%, OC M 126.0 ± 4.8%, OC L 118.1 ± 5.2%, NS by repeated measures ANOVA). Venous compliance (derived from the diameter-pressure relationship during thigh cuff deflation from 60 mmHg – 24 mmHg at 12 mmHg $(10\ s)^{-1}$) tended to be greater in OC than NOC women during both phases (OC M 0.336 ± 0.002% $mmHg^{-1}$, NOC M 0.197 ± 0.084% $mmHg^{-1}$ and OC L 0.314 ± 0.233% $mmHg^{-1}$, NOC L 0.215 ± 0.077% $mmHg^{-1}$) although this did not reach significance. This tendency for greater compliance in the individual venous vessel in OC women is consistent with the findings of Fawer *et al.* (1978) in whole limbs, and may represent modifications of venous tone and/or elastic properties of the vessel wall under the influence of the constituent synthetic hormones within OCs which have a greater binding affinity to receptors than endogenous hormones (Schindler *et al.* 2003).

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PC194

Systemic and local hypoxia may differentially regulate angiogenesis in skeletal muscle

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Hypoxia upregulates a number of pro-angiogenic cytokines, regulated by hypoxia-inducible factor (HIF) (Willam et al. 2002), although in vivo angiogenesis is at best modest. In response to local hypoxia we have previously demonstrated that HIF signalling may be enhanced by preventing its in situ degradation, using the peptide dimethyloxalglycine (DMOG) (Milkiewicz et al. 2004). We examined whether a similar mechanism may operate when the proximate stimulus is systemic hypoxaemia.

Two groups of C57Bl6 mice (n=6 in each) were subjected to 12% normobaric hypoxia, with CO_2 scrubbing. One group received DMOG (Frontier Scientific Europe Ltd) via i.p. injection every other day (8mg in 0.5ml saline) for 14 days. Capillary growth was studied in extensor digitorum longus muscles (EDL) using fluorescein conjugated Griffonia (bandeirea) simplicifolia lectin staining of 10μm cryostat sections, to estimate changes in capillary to fibre ratio (C:F). The present study failed to demonstrate any significant angiogenesis in response to chronic environmental hypoxia. Interestingly, it also showed no effect of HIF stabilisation. Thus, systemic and local hypoxia may act differently, or have different thresholds for inducing capillary growth. As DMOG has a non-specific effect acting on many prolyl hydroxylases in a range of tissues, these data point to the requirement for more targeted pharmacological intervention to enhance angiogenesis under conditions of systemic hypoxaemia.

Table 1. C:F ratio for control, and 2 week hypoxia ± DMOG

Control	Hypoxia	Hypoxia + DMOG	
C:F EDL	1.28 ± 0.05	1.35 ± 0.03	1.37 ± 0.03
EDL mass (mg)	9.1 ± 0.36	10.0 2.81	10.2 ± 2.44
Body mass (g)	26 ± 2.1	26 ± 0.52	27.3 ± 0.61

All values are mean ± S.E.M.

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PC195

Oxygen free radicals and cyclo-oxygenase products: mechanisms underlying Primary Raynaud's disease

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Primary Raynaud's (PR) disease is a disorder of the cutaneous blood vessels, particularly of the fingers and toes such that

they show vasospasm in response to cold or emotional stress. Hyper-reactivity of the sympathetic nervous system or a 'local fault' at the level of the blood vessels, have been suggested to account for PR (Belch, 1990). However, the underlying mechanisms are still not clear. In previous studies, we proposed that vasoconstrictor cyclo-oxygenase (COX) products limit dilator responses to acetylcholine (ACh) in pre- and post-menopausal women with PR disease (Easter & Marshall, 2005). Notably, in women with PR, COX inhibition with aspirin potentiated responses evoked in the finger by iontophoretic application of ACh. We now hypothesise that in PR patients, the COX pathway is distorted such that it produces vasoconstrictor COX products that include O_2^- (Taddei *et al.* 1998). Thus, we have used Vitamin C to investigate the possible involvement of O_2^-, in limiting finger dilatation in PR disease.

In 7 pre-menopausal women with PR disease and 7 age-matched healthy control women, cutaneous red cell flux (RCF) was recorded from the dorsal surface of 2 different fingers of the left hand before and during microiontophoresis of ACh and the NO (nitric oxide) donor sodium nitroprusside (SNP) during the early follicular phase of their menstrual cycle. ACh or SNP was applied iontophoretically with: (7 pulses of 0.1mA for 20s each followed by 1 pulse of 0.2mA for 20s, at 60s intervals) and (5 pulses of 0.1mA for 20s, followed by 1 pulse of 0.2nA for 20s at 120s intervals), respectively (Hendry & Marshall, 2004). This was repeated 3h after oral administration of the antioxidant vitamin C (1000mg) and then again, 30min after aspirin (600mg). All values are expressed as mean ± S.E.M.

The pulses of ACh produced incremental increases in RCF up to 204 ± 28 PU (perfusion units) in women with PR disease. Vitamin C potentiated the responses (269 ± 45 PU; $P < 0.05$, paired t test) but aspirin had no further effect (231 ± 26 PU). By contrast, SNP evoked increases in RCF (to 85 ± 9 PU) that were not affected by Vitamin C (77 ± 11) or aspirin (82 ± 9). In control women, ACh evoked increases in RCF (to 190 ± 33) or SNP (to 88 ± 15) that were not affected by Vitamin C (178 ± 28; 86 ± 13) or aspirin (183 ± 23; 77 ± 10), respectively. These results support our hypothesis that in PR patients, cutaneous vasodilatation evoked in the finger by the endothelium-dependent dilator ACh is limited by O_2^-, produced by the COX pathway.

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PC196

TPRV channels play a role in mediating myogenic-, agonist- and depolarisation-induced tone in pressurised rat mesenteric arteries

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The role for transient receptor potential channels (TRPCs) in vascular contractility are unclear. This study examines the effects of the non-specific TRPV inhibitor ruthenium red (RuRed) and the TRPV1-specific antagonist capsazepine (Cap) in isolated pressurised rat mesenteric arteries.

Mesenteric arteries were dissected from male Wistar rats. Arteries were pressurised to 60mmHg and checked for leaks. The inner diameter and wall thickness was continually monitored using a video dimension analyser. The effects of RuRed (10 μM) and Cap (30 μM) and RuRed + Cap on responses to: (1) changes in intravascular pressure (myogenicity; 20, 40, 60, 80, 100 and 120mmHg); (2) phenylephrine (10 nM-0.1 mM); and (3) 60 mM KCl were studied. Time control responses were examined in separate groups.

The mean diameter (± SEM) of vessels (n=18) used was 214 ± 0.4μm. In vessels which developed myogenic tone Cap alone (n=3) increased active (myogenic) diameter ($P<0.05$, paired t test) at 80mmHg (182 ± 3 μm) when compared to control (160 ± 3 μm). This was not further increased by addition of RuRed. However, in a separate group addition of RuRed alone (n=3) significantly increased ($P< 0.05$, paired t test) myogenic diameter at 80mmHg (206 ± 1 μm) compared to control (170 ± 2 μm). The mean maximum change (± SEM) in diameter (Δmax) to phenylephrine (n=3) in the presence of Cap (89 ± 1 μm) was significantly reduced ($P < 0.05$, paired t test) compared to that obtained in controls (136 ± 2 μm). This was further reduced by the addition of RuRed (8 ± 1 μm) ($P < 0.05$, paired t test) compared to that obtained in the controls and in the presence of Cap alone. EC_{50}s in the presence of Cap (1.4 μM) and both Cap and RuRed (2.9 μM) were not significantly different from control (1.3 μM). The EC_{50} in the presence of RuRed (n=2) (1.2 μM) was significantly different ($P < 0.05$, paired t test) from control (0.6 μM); however, there was no significant difference in maximal response.

Cap had no significant effect on the mean reduction (± SEM) in diameter to KCl (n=3) (25 ± 2 μm) compared to control (42 ± 1 μm). This was further reduced by addition of RuRed (4 ± 1 μm) ($P < 0.05$ compared to control). RuRed alone (n=2) again produced a reduction (84 ± 3 μm) which was significantly less ($P < 0.05$, paired t test) than control (112 ± 3 μm).

The current study suggests that that TRPV1 channels are involved in the myogenic response of pressurised rat mesenteric arteries and in responses to phenylephrine- and KCl-induced tension. Further study is required to elucidate the roles of other TRPV channels.

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PC197

Arteries of the common european frog (*Rana temporaria*) demonstrate arterial myogenic behaviour

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Mammalian small arteries exhibit pressure-dependent myogenic behaviour characterised by an active constriction in response to an increased transmural pressure or an active dilatation in response to a decreased transmural pressure. Such responses are more apparent in distal than proximal branches. This study aimed to determine whether such responses are also a feature of amphibian arteries which are part of a low pressure circulatory system. Arteries from the common european frog (*Rana temporaria*) of either sex were used (body weights 12-37g). Following killing, arteries were dissected free and cannulated at either end with two fine glass micropipettes (prefilled with Ringer solution) in the chamber of an arteriograph (Living Systems). The arteries were pressurised to 20mmHg in the absence of lumenal flow and superfused with Ringer solution at room temperature gassed with 5% CO_2 in air. Large and small mesenteric and sciatic arterial branches were used. The diameters in Ca^{2+}-free Ringer at 20mmHg (μm, mean±SEM (n)) were 345±31 (9) and 179±6 (13) for mesenteric branches and 490±11 (10) and 202±18 (10) for sciatic arterial branches. Arteries were then subjected to incremental increases in transmural pressures (5-40mmHg) in the presence and then in the absence of calcium.

Large arterial branches dilated with increasing transmural pressure and contractile responses were not observed subsequent to step increases in pressure. However, pressure-dependent tone was observed in large mesenteric branches in the range 15-40mmHg and in sciatic branches in the range 25-30mmHg as evidenced by narrower diameters (paired t test followed by False Discovery Rate procedure for multiple comparisons) in the presence of Ca^{2+}. For example, at 30mmHg large mesenteric arterial diameters were 337±32μm and 354±32μm in the presence and absence of Ca^{2+}, respectively ($p<0.05$); equivalent values for large sciatic branches were 507±11μm and 522±13μm ($p<0.05$). A clear myogenic response to a step increase or decrease in pressure was observed in small arteries (5 mesenteric and 6 sciatic vessels). At 30mmHg small mesenteric arterial diameters were 161±9μm and 183±6μm in the presence and absence of Ca^{2+}, respectively ($p<0.01$); equivalent values for small sciatic branches were 187±21μm and 209±19μm ($p<0.05$).

The results demonstrate that (1) amphibian arteries generate spontaneous pressure-dependent tone and (2) myogenic contractile behaviour is more apparent in smaller arterial branches. This is the first study to demonstrate myogenic contractile behaviour in arteries of non-mammalian origin.

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

PC198

Recombinant human growth hormone - effects on arterial pulse wave velocity, homocysteine and high sensitivity C-reactive protein in weight training individuals

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This study investigated the short term effects (6 days) of recombinant human growth hormone (rhGH) administration (0.056 mg/kg/day) and weight training on arterial pulse wave velocity (APWV), homocysteine (HCY) and high sensitivity C-reactive protein (C-RP) in weight training individuals and matched exercising controls. APWV a non-invasive method for measuring atherosclerotic and hypertensive vascular changes increases with age and atherosclerosis leading to increased systolic blood pressure (BP) and increased left ventricular hypertrophy. Aerobic exercise training increases arterial compliance and reduces systolic blood pressure (Kingwell et al. 1997).

Whole body arterial compliance is lowered in strength-trained individuals (Bertovic et al. 1999).

Arterial endothelial dysfunction (an accepted cause of decreased arterial compliance) in growth hormone deficiency is reversed by growth hormone (rhGH) therapy, which favourably influences the risk for atherogenesis (Lilien et al. 2004).

Both HCY and C-RP are two inflammatory markers directly linked with arterial endothelial dysfunction and increased APWV (Bortolotto et al. 1999; Nagano et al. 2004).

The subjects who participated in this study, were twenty four self-prescribing weight lifters (rhGH), aged between 20 and 48 years. Their results were compared with twenty four non-drug using age-matched exercise controls (EC). The dosages were administered under the supervision of the authors (morning administration), before any training sessions. All dosages used were recorded in an administration diary. Group differences were analysed using a two-way (group x time) repeated measures ANOVA at three time points, pre-rhGH administration (day 1), on-rhGH (day 7), post-rhGH administration (day 7). Between group differences were analysed using an independent t test. Within group differences were analysed using a paired t test followed by a post-hoc Bonferroni test. APWV (ms-1), HCY and C-RP concentrations diminished within the rhGH administration group (APWV: 9.97 ± 1.38 vs. 9.18 ± 1.6 vs. 9.26 ± 1.52, ms-1, $p<0.05$; HCY: 13.2 ± 4.0 vs. 11.7 ± 3.1 vs. 13.1 ± 4.3 μmol/l; hsC-RP: 1.77 ± 2.1 vs. 1.29 ± 1.6 vs. 1.7 ± 2.8 mg/l, $p<0.01$) but not in the EC group when the two were compared. RhGH administration resulted in an increase in serum Insulin like growth factor-1 within the rhGH group (159 ± 54 vs. 323 ± 93 vs.175 ± 61 μmol/l, $p<0.001$) and compared with the EC group (323 ± 93 vs. 169 ± 50 μmol/l, $p<0.001$) demonstrating that the growth hormone was responsible for the physiological effects. In conclusion short term use of rhGH altered APWV, HCY and hsC-RP favourably, opening up a series of ethical dilemmas on management of the somatopause.

All data are means ± SD.

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PC199

Different signalling pathways for bradykinin- and histamine-mediated microvascular permeability increase in the isolated perfused rat cremaster muscle

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Bradykinin and histamine have been shown to act as vasodilators via endothelial nitric oxide synthesis and soluble guanylyl cyclase activation in the underlying vascular smooth muscle. As endothelial guanylyl cyclase can also be activated, this could be the mechanism for increased capillary permeability via cGMP formation and PKG activation. We have previously shown that histamine signals permeability increase in this way in pial venular capillaries, but bradykinin uses a different pathway that requires free radicals. We now present data that indicate the bradykinin pathway is not confined to the blood-brain barrier.

The ileal artery of a freshly killed Wistar rat was cannulated orthogradely and branches that did not lead to the cremaster muscle were ligated. The cremaster microcirculation was flushed with St Thomas' cardioplegic solution containing heparin (300 IU ml^{-1}). The scrotum was cut to reveal the cremaster and testes. An incision was made to open the cremaster, care being taken not to damage any major vessel. The muscle was spread over a transparent support and superfused with a Krebs buffer solution maintained at 37°C. The perfusate was changed to a buffer containing albumin (10 mg ml^{-1}) with added FITC-albumin (5 mg ml^{-1}). Perfusate flow was stopped and pressure differences in the vasculature were allowed to dissipate. Permeability was obtained from the rate of change in fluorescent signal across the wall of a selected venule (k) and its diameter (d): P = kd/4.

The resting albumin permeability was $0.30 \pm 0.05 \times 10^{-6}$ cm s^{-1} (mean ± sem, n = 36), which was increased to 2.4 ± 0.22 and 3.6 ± 1.0 (all n = 4) with 1 μM bradykinin and histamine, respectively. The response to bradykinin was blocked by scavenging free radicals with superoxide dismutase and catalase (100 U ml^{-1} each; 0.11 ± 0.05), but unaffected by the nitric oxide synthase inhibitor L-NAME (100 μM; 1.9 ± 0.36). On the other hand the response to histamine was unaffected by superoxide dismutase and catalase (2.0 ± 0.25), but blocked by L-NAME (0.07 ± 0.03). This indicates that the signal transduction in this skeletal muscle is similar to that of the blood-brain barrier. L-NAME application alone increased permeability slightly (0.36 ± 0.07, $p < 0.05$ paired t test), but when co-applied with superoxide dismutase and catalase this permeability change was blocked (-0.10 ± 0.12), which indicates that constitutive NO reduces permeability by scavenging free radicals.

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