201 FOCAL ADHESION PROTEIN AND CYTOSKELETAL REMODELLING IN NORADRENALINE AND ENDOTHELIN-1 STIMULATED VASCULAR SMOOTH MUSCLE CELLS

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Background Focal adhesions (FA) are dynamic transmembrane protein complexes serving as mediators of signalling between the extracellular matrix (ECM) and the actin cytoskeleton (CK). Remodelling of FAs and the CK play key roles in some of the pathological functions of vascular smooth muscle cells (VSMC) such as proliferation, migration and contraction. Focal adhesion proteins (FAPs), constituents of FAs, indirectly dictate some VSMC functions. The FAPs, paxillin, Hic-5 and vinculinâ€™s presence within FAs is well established in VSMCs, however, their behaviour in response to biomechanical and vasoconstrictor stimuli are not. Endogenous vasoconstrictors such as Endothelin-1 (ET-1) and Noradrenaline (NA), alongside changes in ECM have individually promoted changes in the VSMC CK and FA arrangement. To contextualise these cytoskeletal changes and their consequences on vascular function, more information is needed about FAP localisation and CK arrangement in response to vasoactive stimulation alongside ECM changes.
Aims To investigate actin CK remodelling and the localisation of Hic-5, vinculin and paxillin in response to changes in substrate composition and contractile stimulation.
Methods Rat VSMCS (RVSMCs) were cultured on glass or type I collagen-coated glass and were stimulated with 15Î¼M NA, 100 nM ET-1 or remained unstimulated. Cells were stained for actin filaments and either Hic-5, vinculin or paxillin. Changes in CK arrangement were visually assessed. The punctate regions of FAPs were quantified using image analysis software.
Results When cultured on glass, Hic-5 and paxillin occupied punctate regions within RVSMC; vinculin was diffusely spread throughout the cell. The punctate regions were principally localised to the ends of actin stress fibres. Compared to unstimulated RVSMCs (punctate region mean area, 1.043Î¼mÂ²), NA caused a reduction in paxillin punctate region area of RVSMCs cultured on both glass (0.5548Î¼mÂ²; $P < 0.0001$) and collagen (0.7060Î¼mÂ²; $P < 0.001$). NA also induced cytosolic dissemination of Hic-5 without affecting punctate region area. Vinculin localisation did not change in response to NA ET-1 or collagen. ET-1 did not induce changes in paxillin or Hic-5 localisation or CK arrangement. RVSMCs cultured on glass showed a peripheral arrangement of the CK within the cell compared to those cultured on collagen, irrespective of stimulation.
Conclusion The study demonstrates that NA selectively regulates FAP localisation for paxillin and Hic-5, but not vinculin. As ET-1 does not regulate FAP localisation; these results indicate that individual FA remodelling is agonist specific within VSMCs. Furthermore, ECM composition is vital in CK reorganisation in a manner independent of NA-induced FA remodelling. Accordingly, actin CK and FA reorganisation in response to altered ECM composition or vasoconstrictors may contribute to vascular remodelling in cardiovascular disease.

202 AMPK ACTIVATION PARTIALLY RESTORES THE ANTI-CONTRACTILE EFFECT OF PERIVASCULAR ADIPOSE TISSUE IN FEMALE OFFSPRING OF OBESE RATS

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Introduction Maternal obesity programmes offspring to develop obesity and associated cardiovascular disease. Perivascular adipose tissue (PVAT) exerts an anti-contractile effect in healthy blood vessels; an effect lost in male offspring of obese dams. However, the mechanism by which maternal obesity programmes PVAT dysfunction in offspring remains unknown.
Methods Six week old female Sprague-Dawley rats were fed a control (10% fat) or 45% fat obesogenic diet (HFD) for 12 weeks before mating; during pregnancy and lactation. At weaning, female offspring were provided with the control diet until sacrifice at either 12 (12 wo) or 24 (24 wo) weeks of age. PVAT-denuded mesenteric arteries from pups, with or without exogenous PVAT, were mounted on a wire myograph and concentration-response curves were constructed to thromboxane A_2 receptor agonist U46619 (10 nM-3 μM) ± 10 μM A769662, an activator of AMP-activated protein kinase (AMPK) ± glucosamine (an O-GlcNAcylator). Western blotting was used to asses protein expression in PVAT stimulated with or without glucosamine.
Results Body weight, insulin levels and blood pressure were increased in HFD dams and their 12wo and 24 wo offspring compared to age-matched controls. Without PVAT, vessel contractions to U46619 were reduced in 12 wo offspring of HFD dams, effects mimicked in control arteries by pre-incubation with 10 mM glucosamine. PVAT from control, but not from HFD offspring, exerted an anti-contractile effect on the corresponding PVAT-denuded arteries at both ages. Pre-incubation of control PVAT with glucosamine diminished the anti-contractile effect at both ages. PVAT from HFD offspring pre-incubated with glucosamine had no effect on PVAT-denuded vessels but simultaneous AMPK activation within PVAT partially restored anti-contractile capability at both ages. Protein O-GlcNAcylation expression was increased in HFD PVAT and control PVAT incubated with glucosamine, whereas AMPK expression was decreased.
Conclusions The enhanced protein O-GlcNAcylation and decreased AMPK expression in HFD PVAT may underlie the reduced anti-contractile effect of PVAT in female offspring of obese dams. Nevertheless, simultaneous AMPK activation within HFD PVAT partially restored the anti-contractile effects of PVAT.

203 ELN GENE: UKGTN SERVICE FOR SVAS AND CUTIS LAXA. COPY NUMBER VARIANTS (CNVS) ARE A COMMON CAUSE OF DISEASE

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