(PVAT: 81 (20)% vs no PVAT: 140 (27)% at 10^{-5} M NE). The anticontractile properties of PVAT are abolished (n=6) in the presence of aldosterone (PVAT: 53 (24)% vs no PVAT: 62 (35)% at 10^{-5} M NE). Aldosterone significantly reduced tension ($p<0.05$) compared with no PVAT controls. The anticontractile properties of PVAT (n=6) are absent in arteries from obese animals (PVAT: 105 (33)% vs no PVAT: 91 (15)% at 10^{-5} M NE); however, in the presence of aldosterone (n=6) constrictions are significantly increased ($p<0.05$) in arteries with (PVAT: 133 (49)% vs no PVAT: 89 (18)% at 10^{-5} M NE).These results demonstrate that obesity and aldosterone impair the anticontractile effects of PVAT. Aldosterone reduces contractility in healthy arteries but increases contractility in obese arteries.

BAS/BSCR36 REGULATION OF HUMAN SMOOTH MUSCLE CELL DEVELOPMENT BY MYOCARDIN

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Background Myocardin is a cardiac-specific and smooth muscle cell (SMC)-specific transcription factor with a key role in development. It regulates a wide variety of SMC-specific contractile markers by acting as an accessory protein for serum response factor, binding to its DNA binding sites (CArG boxes), and so has a major effect on SMC phenotype. However, its precise role in SMC development remains unclear. Moreover, there are no data on its requirement in human SMC development.
Methodology We investigated whether myocardin was required for SMC development from human embryonic stem cells in an embryoid body model and whether we could promote SMC development at high efficiency by overexpressing myocardin.
Results & conclusions Embryoid bodies from human embryonic stem cells were found to express increasing quantities of SMC markers up to 60 days of development with the appearance of visibly contractile SMC patches as a late phenomenon. Overexpression of myocardin using an adenovirus vector increased differentiation of pluripotent cells into the SMC lineage, although only a subset of cells was susceptible to the overexpressed transcription factor. Loss of function studies using a truncated myocardin dominant negative construct resulted in only minor or no reduction in SMC differentiation, suggesting that redundant pathways exist during embryonic development in human cells.

BAS/BSCR37 DISCOVERY AND CHARACTERISATION OF NOVEL PEPTIDE AGONISTS AND THE FIRST ANTAGONIST FOR THE CARDIOVASCULAR PEPTIDES, THE APELINS

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The recently discovered apelin family of peptides mediate their actions by a single G-protein coupled receptor, APJ. We have previously shown that apelins have three major actions in the human cardiovascular system: endothelium-dependent vasodilatation; direct vasoconstriction by interacting with smooth muscle APJ receptor and increased cardiac contractility by action on cardiac myocytes. Our aim was to discover shorter sequences of (Pyr1)apelin-13 retaining agonist activity and use a computational ligand-based strategy to design cyclic peptide agonists and antagonists. Over 50 compounds were synthesised and tested in a competition binding assay. Functional assays for agonists measured vasoconstrictor action in endothelium-denuded human saphenous veins. Antagonist activity was measured in cyclic AMP assays against (Pyr1)apelin-13. Data are expressed as mean±SE, pD2=−log10EC50, Emax=maximum response. The predicted cleavage product of the angiotensin converting enzyme 2 (ACE-2), apelin-13(1−12), inhibited radiolabelled apelin binding and was identified as the shortest sequence potently constricting endothelium-denuded saphenous vein (pD2 9.07%±0.40, Emax 29.30%±9.43% KCl, n=5). The most potent cyclic analogue identified, MM07, inhibited binding with a KD=86%±30 nM, (n=3) and pD2 10.53%±0.24, Emax 21.80%±5.72% KCl, n=3) with a comparable potency and efficacy to (Pyr1)apelin-13 (pD2=8.8%±0.3, Emax 26%±4%, n=15). MM54 inhibited binding, KD=3.42%±0.45 μmol/l, (n=3) and was identified as an antagonist with a pA2 value of 5.9 in a cyclase inhibition assay. The novel cyclic peptide MM07 retains potency and we have identified the first apelin receptor antagonist, MM54 as a pharmacological tool to characterise the apelin system and in the design of small molecule drugs.

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BAS/BSCR38 CYTOKINE PROFILING IN CULTURE REVEALS A PREDOMINANCE OF M1 MACROPHAGE POLARISATION IN SYMPTOMATIC CAROTID PLAQUES

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Rationale Macrophages in atherosclerotic plaques are a heterogeneous population. To uncover signatures of classical (M1) or alternative (M2) macrophage polarisation during plaque instability, we compared cytokine and chemokine production via Luminex profiling in asymptomatic human carotid plaques with carotid plaques in the territory of recent focal neurological symptomatology.
Methodology Carotid endarterectomy specimens were collected from 50 consenting patients (26 symptomatic, 24 asymptomatic). Fresh specimens were divided symmetrically along their long axis, allowing for representative undertaking of both immunohistochemistry and plaque cell culture (Monaco *et al. PNAS USA* 2004;**101**:5634–9). Automated image analysis (Clemex Vision) quantified CD68 staining. Cells were isolated via enzymatic dissociation to produce a viable mixed cell suspension and cultured for 24 h. Macrophage was the predominant cell type in the mixed cell culture. Supernatants were interrogated with a panel of 22 cytokines and chemokines on a Luminex 100 platform.
Results CD68 immunopositivity was significantly higher in symptomatic than in asymptomatic plaques ($p=0.0075$). Luminex detected 17 of the 22 analytes, with a predominance of myeloid-derived cytokines over lymphoid-derived cytokines, in keeping with the predominance of macrophage in culture. Tumour necrosis factor α, interleukin (IL)-1α, IL-1β, IL-6, granulocyte-macrophage colony-stimulating factor, CCL2, CCL5 and IL-10 levels were significantly higher in symptomatic plaques.
Conclusions Our data demonstrate that symptomatic atherosclerotic carotid disease is associated with a cytokine pattern consistent with the predominance of proinflammatory M1-type macrophage polarisation. M2-dependent cytokine IL-10 was also part of this inflammatory response, suggesting macrophage heterogeneity. Our study has implications for future therapeutic and diagnostic applications for high-risk atherosclerosis.