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PDGF regulation of REST mRNA in vascular smooth muscle

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The Repressor Element 1-Silencing Transcription factor (REST) is a negative regulator of genes implicated in variety of diseases including human neointimal hyperplasia (Ooi and Wood, 2007). We have previously reported its role in controlling pro-proliferative genes in vascular smooth muscle and downregulation of REST expression is important for smooth muscle proliferation (Cheong et al, 2005). However the mechanisms regulating REST levels remain unknown.

Human saphenous vein smooth muscle cells (VSMC) were grown from discarded human saphenous veins obtained at coronary artery bypass graft surgery with ethical consent. Data are presented as means ± sem and were statistically analysed by 1-way ANOVA. Statistical significance was defined as a value of $P<0.05$.

We observe that PDGF, a growth factor associated with atherosclerosis and restenosis, significantly inhibited the REST promoter-driven luciferase activity in VSMC by 43.2±11.1% (n=6). Using quantitative RT-PCR, we show that PDGF treatment resulted in a reduction of REST mRNA expression 34.1±7% (n=20-26). Activation of the PDGF receptor initiates MAPK/MEK, PKC, PI3K and calcium-calmodulin signalling (Hughes et al, 1996). Pretreatment with W-7 (a calmodulin antagonist) significantly prevented REST mRNA downregulation by PDGF, suggesting a role for the calcium-calmodulin pathway. Preincubation with KN-62 (a calcium-calmodulin dependent kinase II (CAMKII) inhibitor) attenuated the effect of PDGF on REST mRNA expression. Likewise, KN-93 (another CAMKII inhibitor) but not its control analog KN-92 was effective in preventing REST mRNA downregulation. Pretreatment of VSMC with siRNA against the δ-isoform of CAMKII also significantly prevented PDGF-induced REST mRNA downregulation. Neither PD98059 (a MEK-1 specific inhibitor), calphostin (a PKC specific inhibitor) nor wortmannin (a PI3K specific inhibitor) had an effect on PDGF-induced REST downregulation.

We here identify a PDGF-calmodulin dependent kinase pathway that regulates REST mRNA levels in vascular smooth muscle cells. This is the first pathway to be identified that impacts upon REST expression and marks an important step in understanding the molecular mechanisms controlling vascular smooth muscle proliferation.

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Apelin and coronary vascular resistance in post-ischaemic rat heart

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The highly basic peptide adipocytokine apelin-13 is the ligand for the G protein-coupled orphan receptor APJ (Tatemoto et al., 1998). The Apelin/APJ system is espressed in almost all tissues. On cardiovascular apparatus, apelin produces inotropic effects involving Na^+/H^+ and Na^+/Ca^{2+} exchangers (Szokody et al., 2002) and vasodilatatory effects involving nitric oxide (NO) (Tatemoto et al., 2001). Simpkin et al. (2007) observed that in isolated rat hearts the addition of apelin during reperfusion attenuates ischaemia-reperfusion (I/R) injury. Since global I/R induces myocardial contracture, we wanted to see whether Apelin-induced vasodilation and myocardial protection can counteract the effect of contracture on coronary vascular resistance (CVR).

Method. Thirty eight anaesthetized rats were killed by decapitation. The hearts were excised and perfused at constant flow with oxygenated Krebs–Henseleit buffer. Coronary perfusion and left ventricular pressure (LVP) were recorded. After stabilization, the hearts underwent 30 min of global ischaemia and 20 min of reperfusion. Apelin-13 was given at 0,5 mM concentration.

Five groups of hearts were performed. In Group I (n = 7; control) the hearts underwent I/R only. In Group II (n=7) they received apelin before I/R for 20min. The other groups received apelin during reperfusion for the same duration. While Group III (n=6) received only apelin, the NOS inhibitor L-NNA and the guanylate-cyclase (GC) inhibitor 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxaline-1-one (ODQ) were added in Group IV (n=8) and V (n=6) respectively.

Results. Data are give as means ± SEM. In Group I a 26±11% increase of CVR occurred in reperfusion. Apelin before ischaemia (Group II) caused a 23±7% increase of CVR during stabilization and 80±18% at the end of reperfusion. When apelin was given during reperfusion (Group III) CVR was similar as in Group I (34±7%) and did not change if NO-synthase and guanylil-cyclase were blocked by L-NNA (Group IV, 45±20%) and ODQ (Group V, 42±16%) respectively. During reperfusion end-diastolic LVP was increased 20±9 folds in Group I, 14±4 in Group II and 22±10 in Group IV. It is likely that apelin before ischaemia induces a vasoconstriction by the activation by Na^+/H^+ and Na^+/Ca^{2+} exchangers not only in myocardium but also in coronary smooth muscle cells. During reperfusion myocardial contracture contributed to the further increase of CVR. Given after ischaemia apelin produced a vasodilatation that exceeded the effect of contracture. The absence of any effect of NO blockade suggests that apelin-induced vasodilatation may be mediated not only

by NO but also by some other factors (e.g. hyperpolarizing factor).

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PC25

Cardiac protein thiol modification by 15-deoxy-delta 12, 14 prostaglandin J2

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Cellular redox signalling is in part mediated by post translational modification of proteins by reactive oxygen species, nitrogen species or by products of their reactions. Enhanced lipid peroxidation has traditionally been causatively associated with many diseases; although important roles in redox signalling are now being recognised. Many lipid peroxidation products are reactive and are capable of reacting with and modifying protein thiols. Cyclopentenone prostaglandins (CyPGs) are model examples of reactive oxidised lipids, containing electrophilic carbon centres that allow covalent adduction with target proteins. To determine if lipid-protein adducts form in a cellular setting, a biotinylated derivative of 15-deoxy-delta 12, 14 prostaglandin J2 (15d-PGJ2) (an electrophilic lipid) was used to treat adult rat ventricular myocytes. There was a dose- and time-dependent increase in biotin-15d-PGJ2 labelling which was maximal at 50μM 15d-PGJ2 and after 120 minutes. 15d-PGJ2 treatment of isolated rat hearts decreased coronary perfusion pressure (vasodilation). Mesenteric vessels were treated with biotin-15d-PGJ2 in order to identify proteins that formed an adduct with 15d-PGJ2. These proteins were purified with avidin-agarose and identified by separation of tryptic digests by liquid chromatography with online analysis by mass spectrometry. Several proteins were identified that formed an adduct. However one particular protein that was modified, soluble epoxide hydrolase (sEH), we hypothesized might account for the vasodilation observed in isolated hearts. sEH catalyses the hydroysis of epoxides, such as epoxyeicosatrienoic acids (EETs), to diols (dihydroxyeicosatrienoic acids). We assayed sEH activity, and found 15d-PGJ2 inhibited its activity, both in vitro with recombinant sEH, as well in cardiac homogenates after hearts were exposed to this lipid. Furthermore, when an inhibitor of sEH, (AUDA), was perfused through isolated rat hearts, it mimicked the vasodilatory response of 15d-PJ2. Similarly, 14, 15-epoxyeicosatrienoic acid (14, 15-EET) also decreased coronary perfusion pressure. These results suggest that 15d-PGJ2 is able to form an adduct with sEH, which inactivates it. This results in cellular accumulation of 14, 15-EET, allowing them to exert their vasodilatory response.

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Expression and role of RhoA/ROK pathway in control of agonist-induced Ca^{2+} signaling in endothelial cells of intact rat tail artery

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The endothelium is a confluent monolayer, lining the inner surface of blood vessels which acts as the main local regulator of vascular wall homeostasis. Binding of inflammatory mediators to G-protein coupled receptors increases endothelial cells permeability by increasing intracellular Ca^{2+} concentration. Several studies have shown that RhoA/Rho-kinase plays a key role in modulation of barrier properties of cultured endothelial cells and intact microvessels [W-S. Beata *et al*, 1998: C.M. Joes *et al*, 1999: R.H. Adamson *et al*, 2002]. Therefore we investigated role of Rho-A and its main effector, Rhokinase in control of calcium signaling in intact endothelial cells of rat tail artery using immunohistochemistry and confocal imaging. Rats were humanely killed under CO_2 anaesthesia; their tail removed from the ventral grove, cleaned of fat and loaded with Fluo-4 AM (Molecular Probes, 15um) with pluronic. Confocal imaging was done using Nipkow disc based confocal imaging system (Ultraview Perkin Elmer, UK). Minimum of 3 animals were used in each set of experiments. We have found that in endothelium of rat tail artery ROK-α but no ROK-β was expressed. Carbachol a muscurinic receptor agonist was used in our study to stimulate the intact endothelial cells. Application of carbachol (0.1uM, 1uM, 10uM) to intact endothelial cells produced a calcium transient which consisted of two components: initial fast - dependent on Ca^{2+} release and subsequent, sustained dependent on Ca^{2+} entry. Sustained component of CCh induced Ca^{2+} transient was 41 $\pm$ 0.7 of the peak taken for 100% (n= 372cells, 7vessels). The frequency of oscillation ranges from 0.05 to 0.3 Hz (n=372cells, 7vessels). Inhibition of Rho-A by C3-transferase (1μg/ml) or Rho-kinase by H-1152 (200 nM) reduced the initial fast component of CCh induced Ca^{2+} transient to 55$\pm$1.5 (n=134 cells, 3 vessels) and 52$\pm$2.1 (n=136 cells, 3 vessels) of the peak, respectively and either fully abolished or significantly decreased the amplitude and the duration of the Ca^{2+} oscillations while the amplitude of the sustained component expressed as a percentage of peak was 65$\pm$1.8% (n=134cells, 3vessels) and 29$\pm$1.6% (n=136cells, 3vessels), respectively. Taken together, the data obtained suggest that Rho-A/ROK pathway is involved in control of calcium signaling induced by CCh in intact endothelium of large conduit arteries.

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