to date have taught us that to understand the rapid vascular mechanisms of steroids, one must (i) know which vascular 'compartment' the steroid is acting and (ii) know which receptor the steroid hormone is activating; and (iii) not assume the receptor specificity of a steroid receptor ligand based solely on its selectivity selectivity for its traditional 'transcriptional' steroid receptor.

042
ABERRANT REGULATION OF THE NA+/H+ EXCHANGER EXACERBATES DAMAGE TO THE MYOCARDIUM IN HEART DISEASE

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The Na+/H+ exchanger isoform 1 (NHE1) is a plasma membrane pH regulatory protein that removes one intracellular H+ in exchange for an extracellular Na+. It has a 500 amino acid N-terminal membrane domain and a 315 amino acid, C-terminal cytosolic domain. In heart disease, ischemia/reperfusion activates NHE1 and this leads to excess sodium uptake which leads to excess intracellular calcium via the sodium/calcium exchanger. We determined that Ser770 and Ser771 were necessary for NHE1 activation. To determine how phosphorylation activates the protein we studied the conformational changes on the regulatory tail. Tryptic digestion of the C-terminal regulatory region showed that a phosphomimetic protein had altered conformation. Tryptophan fluorescence indicated that wild type and phosphomimetic purified C-terminal region had pH-dependent differences in the conformation. Bottom-up hydrogen/deuterium exchange mass spectrometry demonstrated that a peptide fragment containing phosphomimetic mutations became strongly stabilized relative to the wild type protein. The results show that phosphorylation of S770/S771 changes the conformation of the C-terminal regulatory region in a pH-dependent manner, resulting in a more compact region that increases NHE1 activity in the diseased myocardium.
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043
EX VIVO PERFUSION AS A METHOD FOR DONOR HEART TREATMENT AND ASSESSMENT

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Cardiac transplantation remains the gold standard therapy for the definitive treatment of end-stage heart failure. However, application of this therapy is limited by a small number of good quality donor organs. Ex vivo organ perfusion has been proposed as a method to expand the pool of donor organs for transplantation. Devices have been developed and are currently either in routine clinical use, as in the case of kidney, or are under clinical investigation as in the case of lung and liver. We have developed a device that allows full biventricular working mode analysis of donor cardiac function. This device is fully self-contained, eliminating the need for separate dedicated pressure-volume (PV) loop analysis equipment. The utility of the device to accurately and reproducibly assess function was confirmed through comparative analyses with standard PV loop equipment. We then demonstrated the need for functional analysis, as other parameters such as lactate, do not correlate well with myocardial performance. Optimal conditions for normothermic ex vivo preservation of the donor heart, including hemodynamic parameters and perfusate composition are being determined. In summary, ex vivo heart perfusion show great promise as a method for increasing the number and quality of donor hearts for transplantation.

044
NOVEL TARGETS AND THEIR POTENTIAL FOR METABOLIC SYNDROME

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Metabolic syndrome is a cluster of risk factors like central obesity, hypertension, impaired glucose tolerance, insulin resistance and dyslipidemia. It is on the rise globally, and due to incompletely revealed underlying pathophysiology of complications, no standard therapy is yet available. Alterations in fat metabolism, oxidative stress and inflammatory changes could be a few of the major contributing factors of metabolic syndrome. Many molecular targets are studied to explore the underlying mechanisms in this regard, like NFB, Sterol response element binding protein 1-c, Nuclear factor E2 related factor -2, Kelch-like ECH associated protein 1, PPAR-γ, fatty acid binding protein 4, cluster of differentiation 36, LPL, Steroyl-CoA desaturase, Acetyl-CoA carboxylase 1, etc. Metabolism of substrates of CYP450 isoenzymes is also reported to be modulated during various metabolic disorders. Expression of CYP2E1, CYP1A2, CYP2C9, CYP2C19, CYP3A4 and CYP2D6 are reported to be altered in obese condition. Further, disruption of CYP4A14 gene has shown to cause hypertension resembling human hypertension and inhibitors of CYP4A family may have therapeutic benefit in management of diabetic nephropathy. CYP450 is expected to be a novel and potential target; modulation in its expression can be beneficial in prevention of metabolic syndrome and its complications.

045
ROLE OF THE PARASYMPATHETIC NERVOUS SYSTEM IN CARDIOPROTECTION BY REMOTE HINDLIMB ISCHAEMIC PRECONDITIONING

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The goal was to determine the participation of the vagus nerve and muscarinic receptors in the remote ischaemic preconditioning (rIPC). New Zealand rabbits were anaesthetized, and the femoral artery was dissected. The hearts were isolated and subjected to 30 min of ischaemia and 180 min of reperfusion (non rIPC group). In the rIPC group, three cycles of hindlimb ischaemia (5 min) and reperfusion (5 min) were performed, and the same protocol as that used in non-rIPC group was then repeated. The femoral and sciatic nerves were sectioned and in other group the spinal cord was sectioned (afferent pathway). The vagus nerve was also sectioned and, in another group, atropine was administered (efferent pathway). The effect of vagal stimulation was also evaluated. Infarct size was 40.8±3.1% in the non-rIPC group and 16.4±3.5% in rIPC ($P<0.05$). During the rIPC protocol, the vagus nerve section and atropine abolished the effect of rIPC on infarct size. Vagal stimulation also decreased infarct size to 15.2±4.7% ($P<0.05$), and the spinal cord section completely abolished the effect of rIPC. Thus, the cardioprotective signal reaches the heart through the vagus nerve, and muscarinic receptors activate ischaemic preconditioning.

046
EXTENSIVE AUTOLYTIC FRAGMENTATION OF MEMBRANOUS VERSUS CYTOSOLIC CALPAIN FOLLOWING MYOCARDIAL ISCHEMIA–REPERFUSION (I/R)

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Calpain activation was investigated following I/R of rat hearts by Western blot detection of its own autolytic and calpain substrate fragmentation in cytosolic and membrane fractions. After I/R, the catalytic calpain subunit was autolytically fragmented to 56 and 43 kDa peptides primarily in membrane fractions. This accompanied calpain-like degradation of membrane-associated 240 kDa α-fodrin to 150/145 kDa signature peptides. In contrast, cytosolic calpain remained largely intact with only weak immunostaining of autolytic peptide fragments. Myofibrillar α-fodrin was degraded but without a characteristic calpain-induced degradation pattern. In purified SR membranes, RyR2 and SERCA2 proteins were also highly degraded again without production of calpain-induced signature peptides. When I/R-treated hearts were perfused with peptidyl calpain inhibitors, calpain autolysis and α-fodrin degradation were robustly attenuated. However, inhibitors only weakly protected against early loss of developed pressure following I/R. Our data suggest calpain is preferentially activated at membranes following I/R but it may only contribute a small amount to IR injury.
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