racic aorta were then removed and subsequent aortic ring vasorelaxation response to acetylcholine (ACh) was measured with and without L-NMMA, an inhibitor of NO synthesis. Endothelial NO synthetase (eNOS) protein levels were measured from the harvested left ventricles using standard immunoblot techniques. MI rats treated with spironolactone had a decreased (P<0.05) systolic blood pressure (SBP, 128 ± 22 vs 111 ± 10 mmHg), and mean arterial pressure (MAP, 111 ± 23 vs 92 ± 11 mmHg) compared to MI untreated rats. There was no difference in left ventricular (LV) dP/dt (LV dP/dt, 5600 + 1889 vs 4681 ± 696 mmHg/sec) and LV end diastolic pressure, LVEDP (LVEDP, 16 ± 7 vs 21 ± 6 mmHg) in MI untreated animals compared to MI rats treated with spironolactone. Compared to MI untreated rats, spironolactone improved (P<0.05) endothelial dependent vasorelaxation at a concentration of 10^{-7} M ACh and greater. This improvement in endothelial dependent vasorelaxation was attenuated in the presence of L-NMMA. Endothelial NOS protein levels were attenuated (P<0.05) in MI (48.7 ± 17 vs 15.1 ± 7 intensity units/µg tissue) compared to sham rats. MI rats treated with spironolactone had an increase (P<0.05) in eNOS protein levels (42.8 ± 5 vs 15.1 ± 7 intensity units/µg tissue) compared to MI untreated. Our study demonstrate that spirolactone improved NO mediated endothelial dependent vasorelaxation in heart failure by increasing eNOS protein levels.

1024-111 Evaluation of the Porcine Ameroid Constrictor Model of Chronic Myocardial Ischemia for Therapeutic Angiogenesis Studies

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Background: The porcine model of chronic myocardial ischemia is widely used for preclinical testing of angiogenic genes and proteins. Important characteristics of this model like the impact of target vessel occlusion on the presence of myocardial ischemia as well as the relation between morphological, functional, and hemodynamic measurements, however, have not been described in detail.
Methods: We performed a systematic analysis of 94 study animals undergoing ameroid constrictor placement around the left circumflex coronary artery (LCX). Pigs underwent a comprehensive evaluation including echocardiography, coronary angiography and myocardial blood flow measurements at rest/ stress 26±5 days after ameroid placement.
Results: Complete occlusion of the LCX was observed in 34/94 (36%) animals who demonstrated myocardial ischemia of the lateral wall at rest and under stress conditions. By applying a set of angiographic criteria (TIMI<2 flow in LCX or collateral flow Rentrop class >1) another 29% (27/94) of study animals with myocardial ischemia under pharmacological stress conditions could be identified. Echocardiographic parameters of regional and global myocardial function were not associated with myocardial blood flow or the level of ischemia. There was, however, a strong correlation between fractional shortening and the left atrial pressure as a surrogate for cardiac preload (R= -0.36, p=0.005). There was no relation between the extent of coronary collateralisation as assessed by angiography and echocardiographic parameters or myocardial blood flow.
Conclusion: Occlusion of the ameroid instrumented coronary artery is not a pre-requisite for successfully establishing the pathophysiology of chronic myocardial ischemia. The above defined angiographic criteria are useful in identifying animals with appropriate ischemia, despite incomplete LCX occlusion. The left atrial pressure as a surrogate of cardiac preload serves as a valuable predictor of regional myocardial function.

1024-112 Diabetes, Hyperglycemia, GIK, and Cardioprotection: Do ATP-Regulated Potassium Channels Play a Role?

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Background: Insulin is protective, whereas, hyperglycemia is deleterious during myocardial ischemia and reperfusion injury. Diabetes and hyperglycemia attenuate, but insulin activates ATP-regulated potassium (K_{ATP}) channels. We tested the hypothesis that blockade of K_{ATP} channels with pharmacological antagonists or hyperglycemia before ischemia abolishes reductions of myocardial infarct size produced by GIK on reperfusion.
Methods: The temporal dependence of cardioprotection was investigated in barbiturate-anesthetized dogs (n=7 in each group) randomly assigned to receive GIK (25% dextrose; 50 IU insulin/L; 80 mM/L KCl infused at 1.5 mL/kg/hr) starting 75 minutes before coronary artery occlusion or five minutes before reperfusion. The role of K_{ATP} channels in the cardioprotective effects of GIK were evaluated in dogs pretreated with glyburide (0.1 mg/kg iv). The efficacy of GIK was further investigated by increasing blood glucose concentration to 100, 300, or 600 mg/dL (intravenous dextrose) or in diabetic dogs (3 weeks after alloxan-streptozotocin).
Results: There were no differences in area at risk (AAR) or collateral blood flow among groups. Myocardial infarct size (triphenyltetrazolium staining) was 28±2% of the AAR in control dogs. GIK significantly (P<0.05) decreased infarct size when administered at reperfusion independent of blood glucose concentration (13±2 and 12±2%; 100 and 600 mg/dL, respectively). The protective effects of GIK upon reperfusion were abolished in diabetic animals (25±3%), animals receiving glyburide (30±5%), and in those subjected to hyperglycemia before ischemia (27±4%; 600 mg/dL). GIK did not protect against infarction when administered before ischemia (31±3, 27±2 and 35±3% during blood glucose concentrations of 100, 300, and 600 mg/dL, respectively).
Conclusion: The insulin component of GIK and not glucose is responsible for reductions of infarct size when GIK is administered during reperfusion. This action is K_{ATP}-dependent and blocked by glyburide. In contrast, glucose decreases K_{ATP} channel activity, and this effect predominates over that of insulin if hyperglycemia is present before ischemia.

1024-113 Ozone Reduces Reperfusion Injury in an Isolated Rat Heart Model

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Background: Ozone has been used for various clinical conditions associated with ischemia, inflammation or infection. Although there are numerous reports claiming beneficial effects, administration of ozone has remained largely in the realm of alternative medicine. We studied the effects of ozone on reperfusion injury in an isolated rat heart model.
Methods: Twenty six Sprague- Dawley rat hearts randomized to control (n=13) or treatment group (n=13) were perfused with modified Krebs-Henseleit buffer at 37 degrees centigrade and a constant pressure of 90 cm H2O. A latex balloon tipped catheter was inserted into the left ventricular cavity to assess contractile function. After 15 minutes of perfusion, the hearts were subject to 30 minutes of ischemia, after which reperfusion was initiated for 40 minutes during which data was collected every 10 minutes. Hearts with the following pre-ischemic parameters were excluded: heart rate <200; or left ventricular developed pressure (LVDP) (calculated by end systolic pressure minus end diastolic pressure) <80 or >250. Thus there were 8 in the control group and 11 in the treatment group. Ozone was produced in a Dr. Hansler generator PM84 at a concentration of 30 ucgr/cc, and administered in the treatment group after 5 minutes of reperfusion, in distilled water via a sidearm, at a rate of 0.17 cc/minute for a total of 0.85 cc. Heart rate, coronary flow, dP/dt max, and LVDP were measured.
Results: There was no difference in preischemic baseline measurements between the two groups. Nor was there any difference in coronary flow. Hearts perfused with ozone exhibited a significantly better post-ischemic recovery: 61% vs 44% in controls (p=0.01).
Conclusions: Our results show a beneficial effect of ozone in reperfusion of an isolated rat heart model. This is possibly due to a scavenging of free radicals, thus reducing reperfusion injury. Further studies in a large animal model are warranted in order to determine the therapeutic potential of ozone in the setting of myocardial ischemia.

1024-114 In Vivo Myocardial Gene Transfer of Dominant Negative IKK-β Reduces Injury in Ischemia-Reperfusion but Not Straight Infarction

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Background: NF-κB transcription factors drive expression of many genes involved in inflammation and cell survival, both important in ischemia-reperfusion (IR) injury. IKK-β can mediate NF-κB activation through phosphorylation of IκB, however, alternative pathways of activation exist.
Methods: To test the role of IKK-β signaling in cardiac injury, we performed cardiac gene transfer of dominant negative IKKβ (dnIKK-β) in rats 48 hr prior to IR (30 min I; 24 hr R) or infarction without reperfusion (MI).
Results: We found that adenoviral gene transfer resulted in regional transgene expression comprising ~60% of the ischemic area. Ad.dnIKK-β reduced IR-induced NF-κB translocation and IκB-α degradation, and blocked induction of the NF-κB-dependent inflammatory chemokine, MCP-1, in the ischemic area compared with Ad.EGFP.β-gal treated rats (p<0.05). The number of infiltrating neutrophils and myeloperoxidase activity in the ischemic area were decreased in Ad.dnIKK-β-treated rats compared with Ad.EGFP.β-gal-treated rats (p<0.05). The ischemic area was not affected by dnIKK-β expression. However, in IR, Ad.dnIKK-β reduced infarct area by 57% compared with Ad.EGFP.β-gal treated rats or buffer alone (p<0.001). In contrast, in straight MI, dnIKK-β did not affect infarct area (p=NS).
Conclusion: In vivo gene transfer of dnIKK-β prevents IR-induced activation of NF-κB. In this setting, abrogation of pro-inflammatory signals appears more important than loss of NF-κB dependent survival factors, resulting in an overall reduction in infarct size. In contrast, in straight MI, IKK-β-dependent signals do not appear to contribute to injury. These data suggest that IKK-β may represent a valuable target for therapeutic intervention in IR injury.

1024-115 A New Model of Coronary Microthrombosis in Rats and the Protective Effect of a New Thrombin Inhibitor

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BACKGROUND AND OBJECTIVE:Minor myocardial infarction after interventional treatment is not uncommon in the clinic.The aim of this study was to develop a new model of minor myocardial infarction based on endothelial damage and thrombotic occlusion in coronary artery, leading to small myocardial infarcts in rats. Moreover, the protective effect of r-RGD-Hirudin, a thrombin inhibitor, was investigated in this model. METHODS: Forty eight male Sprague-Dawley rats were used in the present study. Rats were anesthetized with sodium pentobarbital and ketamine, and 200 ug of sodium laurate was injected into the coronary artery. The thrombus induction and consequent of endothelial damage were examined by histopathological analyses and electron microscope. To investigate the protective effects of r-RGD-Hirudin,1 or 5 mg/kg was administered intraperitoneally 5 minutes after the injection of sodium laurate;the control group was injected saline instead. RESULTS: Three hours after the injection of sodium laurate, microscopic examination of phosphotungstic acid hematoxylin-stained sections (n=8) and Carstairs Stain sections(n=8) revealed that microthrombi containing fibrin strands obstructed the perforating arteries in the myocardium. Under a transmission electron microscope (n=5), endothelial cells appeared exfoliated and the vascular lumen was obstructed by a thrombus composed of degranulated platelets, fibrin, leukocytes, and erythrocytes. Multiple