1381 ANTIMONY ALTERS KINETICS OF CALCIUM TRANSIENTS OF NEONATAL RAT CARDIAC MYOCYTES

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Cardiotoxicity has long been associated with exposure to antimony compounds. Effects in humans exposed to trivalent forms of antimony include hypotension, bradycardia, ventricular tachycardia, electrocardiographic changes and sudden death. The present study describes the effects of overnight exposure to a nonlethal concentration of potassium antimonyl tartrate (PAT) on calcium transients in neonatal rat cardiac myocytes. Isolated cardiac myocytes were cultured on glass coverslips and after 2 days were exposed overnight to 2 μM PAT in medium with 10% serum. Both control and PAT-treated cells were beating and indistinguishable in microscopic morphology. Myocytes loaded with the fluorescent probe fura-2 were paced at 0.5 Hz using field stimulation and $[Ca^{2+}]_i$ monitored on an inverted phase contrast microscope. The calcium transient was expressed as changes in the ratio of fluorescence at 340nm to 380 nm. For each cell, the average $[Ca^{2+}]_i$ transient was obtained from 15 individual transients. This was done for 3–5 cells per treatment in replicate experiments. Transients were characterized by 1) the basal "diastolic" ratio (D); 2) the peak amplitude of the ratio (A); and 3) the decrease in the ratio that follows A, as expressed by the rate constant of decay (k) that was determined by fitting the data to an exponential. Cells treated with 2 μM PAT exhibited a small increase in diastolic ratio (105% of control), a reduction in peak amplitude (73% of control), and a decrease in k (70% of control). Treatment of PAT-exposed and control cells with isoproterenol (10^{-7} M) altered the $[Ca^{2+}]_i$ transient of both groups consistent with a β-adrenergic effect. The effect of PAT on diastolic ratio was maintained (110% of control), whereas A and k were comparable between control and PAT-treated cells. The demonstrated effects of antimony on $[Ca^{2+}]_i$ transients in contracting myocytes could be a factor in the cardiac arrhythmias observed in humans exposed to antimony.

1382 PRINCIPAL COMPONENT ANALYSIS OF ENVIRONMENTALLY-ASSOCIATED ARTERIOSCLEROTIC PLAQUE DEVELOPMENT

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Heart disease is the single greatest killer in the U.S. The cockerel (young rooster) is a reliable animal model for investigating the promotional effects of environmentally-relevant levels of toxicants on arteriosclerotic plaque development, and the mechanism of action of these agents. Our analyses to date have concentrated on the effects of test agents on plaque development in the abdominal aortas from varying sized groups of treated vs. control animals. Comparisons were made on pooled data from each group. We have extended the analysis to compare responses of individual cockerels within and between groups. Data were analyzed from earlier studies with "second-hand" cigarette smoke, putative carcinogens in the smoke, other carcinogens and carbon monoxide as the test agents. Routes of exposure were by inhalation or injection. Principal component analysis revealed that the most important component of the data was the weighted average of plaque size. The most pronounced promotional effect of the active environmental agents was on plaques located in the distal two fifths of the abdominal aorta. The analysis distinguishes clearly between "responders" and "non-responders". The results also show that test groups with as few as ten cockerels each can be a reasonable size to determine whether toxicants accelerate arteriosclerosis at doses which do not cause other overt health effects.

1383 NON-LETHAL ENDOTOXIN (LPS) TREATMENT DECREASES CONTRACTION RATE AND INDUCES NITRIC OXIDE SYNTHASE ACTIVITY IN CULTURED RAT CARDIAC MYOCYTES

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Sepsis and endotoxic shock often result in myocardial depression. Recently, nitric oxide (NO) production by inflammatory cells and other tissues has been proposed to contribute to this phenomenon. In studies to determine the effect of cytokine stimulated NO production on cardiac myocytes, the inflammatory, cytokines TNF, IFN and IL-1 were found to increase nitrite in cell media over 48 h treatment. TNF, IL1, and IL6 increased contraction rates as did the NO synthase inhibitor NMA. Other researchers have shown that LPS-stimulated macrophages co-cultured with myocytes also increased nitrite production and decreased contraction rates. We investigated the effects of non-lethal concentrations of *Escherichia coli* endotoxin (LPS) without added cytokines on cultured cardiac myocytes. Parameters measured included- cytotoxicity, myocyte contraction rate, and NO synthesis as measured by nitrate and nitrite present in the culture media. The concentrations of LPS used were not overtly toxic to cells up to 24 h exposure (as measured by LDH release and cell attachment). Nitrite/nitrate in the media remained low at all concentrations from 0 – 4h and then rapidly increased in a concentration-dependent manner out to 24 h. Myocyte contraction rate followed a similar response pattern in that from 0 – 4h cells contracted at similar rates regardless of LPS concentration. After 6 h, contraction rates were observed to decrease in a time and concentration-dependent manner with contraction rate inversely correlated to media nitrate/nitrite concentrations. In summary, this research demonstrates that LPS-exposed cardiac myocytes generate NO independent of additional cytokine or other inflammatory/immunological stimulus.

1384 APPLICATION OF RT-PCR TO REGIONAL MAPPING OF β_1- AND β_2-ADRENERGIC RECEPTOR mRNAs IN RAT HEART

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Chronotropic and inotropic activities of the heart are stimulated through β_1- and β_2- adrenergic receptors (AR). Regional distribution of β-ARs and corresponding mRNAs in the heart are not well defined despite the potential for molecular phenotypic redistribution to be a factor in pathological conditions. Investigating regional distribution of specific mRNAs in the rat have been hampered by the paucity of tissue and the low abundance in heart of β-ARs. Reverse transcription in combination with the polymerase chain reaction (RT-PCR) was used to measure the number of β_1- and β_2-AR mRNA molecules in the four chambers of rat heart. The right atria was microdissected into four sections with the SA node limited to subsection RA/1. RT-PCR resulted in single amplification products of expected sizes. cRNAs specific for β_1- and β_2-ARs but with restriction sites produced by punctual mutations were used as internal standards. The mean number of β_1-AR mRNA molecules per ng of total RNA ranged from 663 ± 75 to 918 ± 90 in the ventricular and atrial regions. There was significantly more β_1-AR mRNA in right atria than left atria or ventricles. Right atrial subsections RA/1 and RA/2 contained significantly more β_1-AR mRNA than subsections RA/3 and RA/4. The number of β_2-AR mRNA molecules ranged from 375 ± 67 to 481 ± 104 in the regions examined. The number of β_2-AR mRNA molecules did not differ significantly among the chambers or subsections. The sums of β_1- and β_2-AR mRNAs among the individual chambers and atrial subsections were equivalent, but the β_1-/β_2-AR mRNA ratios ranged from 1.45 ± 0.08 to 2.49 ± 0.22 and varied significantly. The highest ratio occurred in the right atria and the lowest in left ventricle. Within the right atria, subsection RA/1 exhibited the highest ratio which was significantly greater than subsection RA/3, but not subsections RA/2 or RA/4. Comparison of the above variables between adult (3 mo) and senescent (24 mo) rats revealed significant phenotypic remodeling of β-AR mRNA distribution throughout the myocardium. These changes could contribute to increased rhythm disturbances observed in senescent heart.

1385 THE RELATIONSHIP BETWEEN PLASMA CONCENTRATIONS, HEMODYNAMIC EFFECTS, AND CARDIOVASCULAR (CV) TOXICITY IN DOGS TREATED WITH MINOXIDIL (MNX)

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The threshold hemodynamic changes associated with MNX-induced CV toxicity characterized by subendocardial necrosis, right atrial hemorrhage, and coronary vascular medical hemorrhage and necrosis in the dog have not been defined. To determine the relationship between plasma concentration, hemodynamic effects [heart rate (HR) and mean arterial pressure (MAP)] and CV toxicity, groups of female Beagle dogs were treated with a continuous IV infusion of dextrose (control), 0.05, 0.14, 0.43, 1.44 or 4.32 mg/kg/day of MNX for 3 days. Plasma concentration of MNX increased in a time-dependent dose related manner. The no significant effects doses (steady-state plasma concentrations of MNX) for hemodynamic effects and CV toxicity were 0.05 mg/kg (2.8 ng/mL) and 0.14 mg/kg (7.6 ng/mL), respectively. CV toxicity occurred at a steady-state plasma concentration of 16.6 ng/mL (0.43 mg/kg) where HR was increased by 51 beats/min and MAP was decreased

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Preface

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An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 351.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 375.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.

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