LB1

Feasibility Of Intravascular Adeno-associated Viral Transduction *In Vivo*

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Adeno-associated viruses (AAV) are attractive vehicles for intravascular gene delivery. By transduction of non-dividing cells and long-term gene expression through integration, they might offer solutions for restenosis, angiogenesis and transplant arteriopathy. We investigated the potential for local intravascular gene delivery using AAV in an *in vivo* rabbit model. **Purpose:** To determine the feasibility of intravascular transduction of vascular cells, we examined the time course of gene expression, the main cell types transduced, the effect of varying viral titers and exposure time and the inflammatory reaction. **Methods:** AAV2 vector with an alkaline phosphatase (AP) reporter gene was injected intralumenally into rabbit carotid arterial segments, transiently isolated between atraumatic clamps. Blood flow was reestablished after exposure times of 30 and 60 minutes. AP gene expression was visualized histochemically at 3, 7, 10, 14, and 28 days post transduction. Viral titers were varied between $5x10^8$ and $2x10^9$ AP FFU. **Results:** Gene expression peaked at day 14 and remained clearly detectable by 28 days. Vascular smooth muscle cells were the main cell type transduced. High titer versus low titer significantly increased expression (33.7 ±6.8% *vs* 15.5±2.6%) ($p<0.02$). Increasing exposure time also enhanced expression (33 ±6.8% vs 21.2±4.1%). Inflammatory reaction was minimal. **Conclusion:** Local *in vivo* intravascular transduction of reporter genes is feasible with AAV vectors. This data is promising for gene delivery applications of AAV in cardiovascular diseases as well as oncology.

LB2

DOWNREGULATION of α_2-ADRENOCEPTORS IN THE NUCLEUS TRACTUS SOLITARIUS BY CHRONIC ETHANOL FEEDING CONTRIBUTES TO ITS HEMODYNAMIC INTERACTION WITH α-METHYLDOPA IN TELEMETERED SPONTANEOUSLY HYPERTENSIVE RATS.

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In this study, we investigated whether chronic ethanol administration attenuates the hypotensive action of acutely administered α-methyldopa in radiotelemetered freely moving spontaneously hypertensive rats, and whether this interaction is a result of downregulation of α_2-adrenoceptors in the nucleus of the solitary tract (NTS). Changes in blood pressure (BP) and heart rate (HR) were evaluated in pair-fed rats receiving liquid diet with or without ethanol for 12 weeks. The hemodynamic effect of subsequent intraperitoneal administration of a single dose of α-methyldopa (100 mg/kg) was also investigated in the two groups of rats. In comparison with pair-fed controls, ethanol feeding (2.5 or 5%, w/v) elicited gradual and dose-related decreases in BP that remained throughout the duration of the study. α-Methyldopa (100 mg/kg, i.p.) produced significant ($P<0.05$) decreases in BP that continued for at least 8 hours. Compared with control rats, ethanol dose-dependently attenuated the hypotensive effect of α-methyldopa. Autoradiographic visualization of α_2-adrenoceptors in brainstem coronal sections with [^{125}I]p-iodoclonidine showed that ethanol feeding significantly ($P<0.05$) reduced α_2-adrenoceptor density (Bmax) in the middle (275 ±28 vs. 173 ± 26 fmol/mg protein) and rostral (126±12 vs. 68 ±12 fmol/mg protein) regions of the NTS. It is concluded that chronically administered ethanol counteracts α_2-adrenoceptor-mediated hypotension, at least partly, through downregulation of α_2-adrenoceptors in the NTS, the main site of the hypotensive action of α-methyldopa.

Supported by Grant AA07839 from the NIAAA.

LB3

EDG G-PROTEIN COUPLED RECEPTORS AND VASCULAR SMOOTH MUSCLE CELLS

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The first member of the Endothelial Differentiation Gene (EDG) family of G-protein coupled receptors (GPCRs) was discovered in human umbilical vein endothelial cells (HUVECs). Subsequently, several other homologous receptors have been identified that are not expressed in HUVECs. The ligands for most members of the EDG family are sphingosine 1-phosphate (SPP) and lysophosphatidate (LPA). We examined the expression profile and potential functions of EDG GPCRs in contractile and intimal vascular smooth muscle cells (VSMCs) from adult and neonatal Wistar Kyoto rats respectively. Northern hybridization analysis revealed expression of EDG1, EDG2, EDG3 and EDG5 in both smooth muscle cell phenotypes. SPP and LPA treatment of the adult VSMCs over four days increased cell number by 20% and 40% respectively. The presence of multiple EDG isoforms in VSMCs and their potential role in SPP and LPA induced proliferation suggest that the functions of the EDG family of GPCRs in VSMCs deserve further study. This research was funded by the NIH and the AHA.

LB4

DEMONSTRATION OF ADENOSINE A_{2A}-RECEPTOR-MEDIATED RELAXATION OF MOUSE AORTA *IN VITRO*.

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Adenosine (Ado), an endogenous vasoactive nucleoside, produces potent vasodilation in the majority of vascular beds of most mammalian species through activation of A_2-receptors. Ado has been shown to cause both endothelium-dependent and independent dilatation in isolated vessels. In this study we have characterized, for the first time, the *in vitro* Ado receptor-mediated vasorelaxation of mouse aorta. For this purpose, concentration-response curves (CRCs) to Ado and 2-chloroadenosine (CAD) were obtained on isolated ring preparations of mouse thoracic aorta precontracted with phenylephrine (1 μM). Mouse aortic rings (3-4 mm in length) were mounted in the isolated organ baths, and changes in the isometric tension were measured using a digital data acquisition system (BIOPAC). The presence of endothelium-dependent or independent vasorelaxation was confirmed by CRCs to acetylcholine and sodium nitroprusside, respectively. The threshold concentration for the vasorelaxant effect of Ado and CAD was at micromolar level, and CAD produced a more complete relaxation. The EC-50 values for Ado and CAD were 210 ± 25 and 72 ± 13 μM, respectively. A selective A_{2A}-receptor antagonist, ZM-241385 (4-(2-(7-amino-2-(2-furyl(1,2,4)-triazolo(2,3-a(1.3,5)triazin-5-yl-aminoethyl)phenol) at 1 μM shifted the CRC for CAD to the right. The results demonstrate an Ado-receptor mediated vasorelaxation in mouse aorta, in part, through activation of A_{2A}-receptors.

Supported by HL 27339.

LB5

ELEVATED GLUCOSE CONCENTRATION INHIBITS NORMAL VASOMOTOR FUNCTION FOLLOWING VASCULAR CRUSH INJURY.

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Background: Angioplasty in diabetic patients has been associated with higher adverse outcomes compared to bypass surgery. To evaluate this, the effect of elevated glucose on arterial vasomotion was tested in an organ chamber. Methods: Fourteen carotid arteries from 7 NZW rabbits were extracted and the whole artery perfused with a physiological buffered solution (PBS) using either 100 or 250 mg/dl glucose concentration. Crush injury was performed with a clamp at three adjacent sites for 1 min each in all arteries and perfusion resumed using PBS at either glucose concentration for 1 hr. Using norepinephrine (NE $1x10^{-6}$ M) preconstriction, pharmacologic challenge with acetylcholine (Ach; $1x10^{-5}$ M) and nitroprusside (SN; $1x10^{-5}$ M) was conducted. Vessel diameter was measured using a computer planimetery system. Results: Lumen diameter change was reported as a percentage difference from NE preconstriction. Relaxation response was significantly reduced after arterial injury at elevated glucose concentration but not before injury. Overall relaxation was reduced after injury at both glucose concentrations ($p<0.01$).

% Lumen Diameter (Relaxation)

[Glucose]	Before Injury (n=7)		After Injury (n=7)	
	(Ach-NE)/NE	(SN-NE)/NE	(Ach-NE)/NE	(SN-NE)/NE
100mg/dl	67.7± 11.2	69.7± 8.8	12.6±2.2*	15.4±2.1**
250mg/dl	62.3±10.5	61.3±11.2	4.3± 0.9*	6.4±1.3**

* p,**p<0.05 (comparison of relaxation after injury at 100 and 250 mg/dl)

Conclusions: These data demonstrate that arterial injury reduces normal vasomotor relaxation responses. However, the presence of high glucose enhances this effect significantly. This is perhaps one of the mechanisms that contributes to the adverse clinical outcomes observed in diabetic patients following angioplasty.

LB6

HEMODYNAMIC LOAD INCREASES G PROTEIN RECEPTOR KINASE EXPRESSION IN LEFT VENTRICULAR VOLUME OVERLOAD,

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We have recently reported that lymphocyte GRK2 and 3 mRNA expression is significantly elevated in association with a decrease in that of the β_2-adrenoceptor (β_2-AR) and receptor-stimulated adenylyl cyclase activity in patients with left ventricular volume overload (LVO). In this study, we evaluated the possibility that LVO induces disease-dependent alterations in the expression of G protein receptor kinases (GRKs). We therefore compared the expression of GRKs 2 - 6 and the β_2-AR in the four myocardial chambers (n = 5) and peripheral lymphocytes (n = 6) of LVO patients with that in control tissues from potential donor hearts (n = 4) or healthy blood donors (n = 9). Quantitative gene expression was determined by RT-PCR followed by densitometric quantification using β-actin as an internal control. All except GRK5 were detectable in both the myocardial and lymphocytes of the control subjects. In this group, GRK5 was well expressed in the myocardium but scarcely detectable in the lymphocytes. Compared to the controls, there was a *de novo* expression of GRK5 in the lymphocytes and an increase of 45% ($p<0.05$) in the right atrium of the LVO group. GRK2 was elevated by 41% in the lymphocytes and by 49% ($p<0.01$) in the right ventricle, while GRK3 was increased by 37% ($p<0.05$) in the lymphocytes and by approximately 15-27% in all four chambers. There was no significant alteration in either the myocardial or lymphocyte GRK4 or GRK6 mRNA expression. These changes were associated with 35% decrease ($p < 0.05$) in the β_2-AR mRNA in lymphocytes, but a less remarkable change in the myocardium of the patients. The alterations in gene expression were not related to the extent of heart failure, but appeared rather to correlate with the changes in the hemodynamic variables, such as ejection fractions as well as the left ventricular end diastolic and systolic dimensions of the patients. The results suggest that increased hemodynamic load in these patients may be primarily responsible for the localized and selective elevation of the GRK mRNAs both in the myocardial chambers and the lymphocytes, and therefore attenuation in β_2-AR signaling.