tive AT2 receptor activation were evaluated in the absence (n=12) and in the presence of NG-nitro-L-Arginine (L-NA; $3*10^{-5}$ M; n=9) or Indomethacin (INDO; 10^{-3} M; n=7), as well as, in muscles without endocardial endothelium (EE; n=7). Calculated parameters: active tension (AT), peak rates of tension rise and decline (dT/dtmax and dT/dtmin, respectively), peak shortening (PS) and peak rate of shortening (dL/dtmax). Results are presented as mean±SEM in % of baseline (p<0.05). **Results:** Selective AT2 stimulation induced a dose-dependent negative inotropic effect, decreasing, at 10^{-5} M of AT-II, 29.3±7.7% AT, 26.1±7.0% dT/dtmax, 27.9±7.5% dT/dtmin, 30.7±9.3% PS and 22.0±5.7% dL/dtmax. This effect was not influenced by L-NA (10^{-5} M of AT-II decreased 32.5±10.2% AT, 25.7±7.8% dT/dtmax, 26.7±8.6 dT/dtmin, 16.90±7.1 dL/dtmax) or INDO (10^{-5} M of AT-II decreased 34.4±7.1% AT, 27.9±6.1 dT/dtmax, 33.2±7.9 dT/dtmin, 20.2±5.0 dL/dtmax, 36.6±10.2 dL/dtmin, 25.3±7.1 PS), but was completely abolished after selective removal of the EE. **Conclusions:** Selective AT2 stimulation induces a negative inotropic effect, which is modulated by the EE, but not mediated by NO or prostaglandins. Such findings might help to better understand the therapeutic effects of selective AT1 antagonists, which are being increasingly used in for treating cardiovascular diseases.

1106-181 Clopidogrel Is an Inducer and a Potent Reversible Inhibitor of Cytochrome P450 3A4 In Vitro

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Background. Inter-individual variability in the response to clopidogrel has recently been described in patients undergoing percutaneous coronary intervention. A recent *ex vivo* study has suggested that: 1) clopidogrel is metabolically activated by cytochrome P450 (CYP)3A4; and 2) clopidogrel may be both an inducer and an inhibitor of CYP3A4. We hypothesize that the observed variability in the antiplatelet response to clopidogrel may be in part due to CYP3A4 induction via the nuclear pregane X receptor (PXR) response element, and/or competitive inhibition of CYP3A4.
Methods. To test whether clopidogrel is capable of CYP3A4 induction in vitro, we used LS174T tumor cell that express CYP3A4, and is inducible by classical PXR ligands. The cells were purchased from the American Type Culture Collection (Manassas, VA), plated, and cultured. Then total RNA was prepared and assayed for CYP3A4 mRNA expression using real-time polymerase chain reaction analysis in the presence of clopidogrel as compared to control. To test for CYP3A4 inhibition by clopidogrel, bacterially expressed human liver CYP3A4 in a reconstituted system was used. Using purified reconstituted CYP3A4 the clopidogrel-dependent inactivation of the testosterone 6 beta-hydroxylase activity of CYP3A4 was investigated. The activity of CYP3A4 was measured by high pressure liquid chromatography. Incremental doses of clopidogrel were added to primary reaction mixtures containing purified CYP3A4.
Results. 1 μM and 10 μM clopidogrel induced CYP3A4 mRNA by 3.9 ± 0.2 and 8.7 ± 0.6 fold as compared with control. No mechanism-based or suicide inhibition of CYP3A4 by clopidogrel was observed (there was no significant loss of CYP3A4 activity following preincubation with clopidogrel and NADPH for 0, 5, 10, and 20 minutes). However, competitive inhibition of CYP3A4 by clopidogrel was observed at concentrations of 2.5 μM and 10 μM of clopidogrel with 61% and 68% of the catalytic activity of CYP3A4 inhibited, respectively.
Conclusion. Variability in clopidogrel response may be in part explained by the *in vitro* findings that clopidogrel is a CYP3A4 inducer and an potent reversible competitive inhibitor of human CYP3A4.

1106-182 Insulin Enhances Vascular Cell Adhesion Molecule -1 Expression in Human Cultured Endothelial Cells: A Link to the Pathogenesis of Accelerated Atherosclerosis in Diabetes

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Hyperinsulinemia is a risk factor for atherosclerosis by mechanisms poorly understood. We assessed if insulin (I) can increase monocytes - endothelial interactions implicated in atherosclerosis. Human umbilical vein endothelial cells were incubated with I for 0 - 24 hours ± tumor necrosis factor (TNFα 0.1 ng/mL), lipopolysaccharide (LPS 0.1 ng/mL), the p38mitogen activated protein(MAP) kinase inhibitor SB203580 (SB 0.1 - 20 μg/mL) and the phosphatidylinositol (PI)3 - kinase inhibitor wortmannin (WT 10^{-9} to 10^{-6}mol/L). Expressions of vascular cell (VCAM -1) or intercellular (ICAM -1) adhesion molecules, and E -selectin were assessed by enzyme immunoassay (EIA), flow cytometry, immunocytochemistry and northern analysis. U937 cell adhesion to endothelial cells was determined by a rotational adhesion assay. At pathophysiological concentrations I induced surface expression of VCAM -1 but not ICAM-1 or E-selectin and potentiated the effects of TNFα and LPS. I 10^{-8} mol/L ± TNF increased U937 cell adhesion by 9.2 and 2.7 fold respectively, and markedly induced expression of VCAM-1 mRNA. In the absence of any cytotoxicity WT 10^{-7}mol/L potentiated the effect of I alone, while SB 1 μg/mL abolished this effect. In the Table p< .05 vs unstimulated control *, control with I #, LPS **, TNF ***, ≠ data lacking because of cytotoxicity. In conclusion I promotes VCAM -1 expression by a p38MAP Kinase pathway amplified by the PI3 -kinase block. This effect may contribute to atherosclerosis in hyperinsulinemic subjects.

Optical density values for VCAM - 1 at EIA (mean ± SD % of unstimulated control)

	I 10^{10} mol/L	I 10^{-9} mol/L	I 10^{-8} mol/L	I 10^{-7} mol/L
I	96±15	130±10*	150±15*	160±13*
I+WT	124±15	180±17*	198±13*	213±10*
I+SB	96±4	118±16#*	98±12#*	102±6*#
I+LPS	139±11	175±15**	235±23**	238±13**
I+TNF	242±25	489±14***	493±20***	≠

1106-183 Adrenomedullin Is a Pulmonary Peptide

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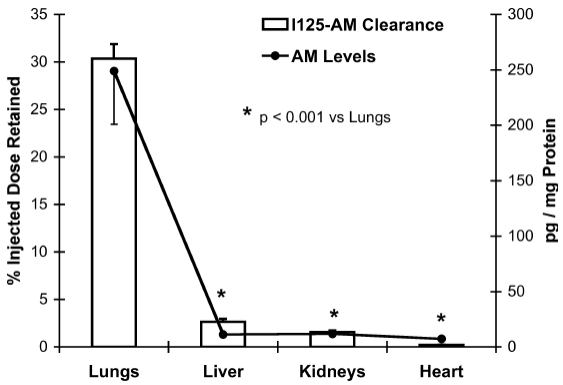
Background: Adrenomedullin (AM) is a multifonctional regulatory peptide highly expressed in endothelial cells. AM levels increase in various conditions, including pulmonary hypertension. This study was designed to quantify the in vivo biodistribution and clearance of AM in rats with special attention to the lungs.
Methods: Plasma kinetics of AM was determined by intra-venous injection of ^{125}I-AM in rats. The retained radioactivity at equilibrium was determined in the heart, lungs, liver and kidneys. First pass pulmonary clearance was evaluated by comparing intra-venous to intra-arterial injections. Arterio-venous plasma and tissue levels of unlabelled AM were determined in another set of rats.
Results: ^{125}I-AM was rapidly cleared from plasma following a two compartment kinetic model with a half-life of 12 minutes. The lungs retained the majority of the injected ^{125}I-AM (figure). There was important first pass pulmonary clearance evidenced by a drop in extraction from 30.4 1.5% after intra-venous injection to 13.5 0.6% after intra-arterial injection. Unlabelled AM tissue levels were also much higher in the lungs (figure), however there was no significant arterio-venous difference of AM in plasma.
Conclusion: The lungs are the major site for AM clearance and production. The lungs could be a preferential target for this peptide and may modulate its circulating levels through reduced clearance and/or increased production.



1106-184 An Antiulcer Drug, Geranylgeranylacetone, Suppresses Nitric Oxide Synthesis in Cytokine-Stimulated Cultured Vascular Smooth Muscle Cells

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Background: Geranylgeranylacetone (GGA) is commonly used as an antiulcer drug. If GGA affects nitric oxide synthesis in the vascular tissue, it could influence disease progression in coronary arteries. We investigated the effects of the antiulcer drug GGA on nitric oxide synthesis in vascular smooth muscle cells.
Methods and Results: Primary cultures of vascular smooth muscle cells were obtained from the media of thoracic aortae of Sprague Dawley rats. We measured the production of nitrite, a stable metabolite of nitric oxide, in cultured rat vascular smooth muscle cells with the Griess reagent. Inducible nitric oxide synthase protein and mRNA expressions were assayed by Western blotting and reverse transcription-polymerase chain reaction, respectively. The levels of NF-kappaB proteins in nuclear extracts were analyzed by gel retardation assay. Heat shock protein 70 (Hsp70), a cytoprotective molecule, was evaluated by Western blotting. Incubation of cultures with interleukin-1beta (10 ng/ml) for 24 h caused a significant increase in nitrite generation. Interleukin-1beta-induced nitrite production by vascular smooth muscle cells was significantly suppressed by GGA in a dose-dependent manner (10^{-8}-10^{-4}M). GGA-suppressed nitrite production was accompanied by decreased inducible nitric oxide synthase mRNA and protein accumulations. GGA by itself did not modulate the basal level of nitrite production. Interleukin-1beta induced NF-kappaB activation in vascular smooth muscle cells, and the addition of GGA further inhibited this NF-kappaB activation. GGA itself induced Hsp70 protein expression in a dose-dependent manner.
Conclusions: These findings demonstrated that GGA suppresses nitric oxide synthesis in cytokine-stimulated cultured vascular smooth muscle cells partially through the suppression of NF-kappaB activation, suggesting that GGA may modulate the pathophysiology of cardiovascular diseases including atherosclerosis. In addition, this effect may be associated with Hsp70 production by GGA.