investigated the role of NO in the development of tolerance to prolonged administration of alpha-2 adrenergic agonists.

**Method:** Tolerance was assessed by the attenuation of the inhibitory effect of dexmedetomidine (DEX), a highly specific alpha-2 adrenergic agonist, on prostaeglandin E-1 (PGE-1) stimulated endothelium derived NO (EDNO). Cells were chronically exposed to 0.1 μM DEX for 2, 8, 14 hours and DEX = 100 μM N-nitro-L-arginine, a NO synthase (NOS) inhibitor, for 2 hours. The cells were also exposed to L-arginine (a NO donor) for 20 minutes. The cells were then washed three times and acutely exposed to varying doses of DEX (1pM to 1 μM) and the AC activity measured.

**Results:** Cells exposed to DEX for 2 hours or longer were desensitized as evidenced by a 10 fold shift to the right of the DEX dose response curve. The cells exposed to DEX and N-nitro-L-arginine for 2 hours showed no such desensitization. Cells exposed to L-arginine (without DEX) demonstrated a 6 fold shift to the right in the DEX dose response curve.

**Summary:** Tolerance to DEX can be induced in an in-vitro model. This is demonstrated by the right shift in the dose response curve, and is more profound when a 24 hour exposure to DEX. This effect can be reproduced by NO exposure after only 20 minutes, and prevented when NOS is inhibited during prolonged DEX exposure.

**M4**

C-Reactive Protein Alters Vascular Reactivity by Increasing Nitric Oxide Production

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**Background:** Increasing circulating concentrations of C-reactive protein (CRP) are predictive of future atherothrombotic events. CRP is present in atherosclerotic lesions, binds to lipoproteins and activates complement, but direct vascular effects of CRP have not been reported.

**Methods:** Endothelium-intact aortic rings from male Sprague-Dawley rats were incubated for 4 h in serum free Dulbecco's modified Eagle's medium containing isolated human CRP (200 mg/l), or its buffer, and then mounted in organ baths. Concentration-response curves to phenylephrine (PE) (10^-10-10^-7 M) and acetycholine (Ach) (10^-10-10^-6 M) were constructed. The effects of endothelial denudation, actinomycin D (10^-6 M; to inhibit protein synthesis), L-NAME (3x10^-6 M, to inhibit NO synthesis), 1400 W (10^-6 M, to selectively inhibit iNOS) or methoxyacetylsertotonin (10^-6 M to inhibit tetrahydrobiopterin synthetin) were also determined. Stock CRP solution and its solvent buffer contained <0.5 mg/ml bacterial lipopolysaccharide (LPS) by Limulus chromogenic assay. To exclude LPS effects and confirm the specific role of CRP, the stock CRP preparation was absorbed before use with Sepharose-phosphoethanolamine beads, reducing the final CRP concentration to 200 ng/ml. Changes in protein levels of eNOS and GT-Pychohydrodrolase-1 (the rate limiting enzyme for tetrahydrobiopterin synthetin) were estimated by SDS-PAGE and Western blotting.

**Results:** Human CRP produced marked hyporeactivity to PE (P<0.0001, 2-way ANOVA) in rat aorta and enhanced relaxation to Ach (P<0.0001). This effect was abolished by specific removal of CRP, by endothelial denudation, by pre-treatment with actinomycin D, and treatment with L-NAME or methoxyacetylsertotonin, but not by L-NAME. Western blots showed an increase in GT-Pychohydrodrolase-1 levels.

**Conclusion:** Human CRP causes hyporeactivity to PE in isolated rat aorta by increasing NO synthesis from eNOS, through a pathway that may involve induction of the eNOS co-factor tetrahydrobiopterin. The pathophysiological significance of these findings is currently being studied.

**M5**

Circulating plasma levels of thrombospondin and cellular adhesion molecules in acute coronary syndromes

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The acute coronary syndromes encompass a spectrum of disorders that present with chest discomfort caused by myocardial ischaemia. Platelet and endothelial activation secondary to plaque instability have been implicated as major pathogenic mechanisms in the acute coronary syndromes. Thrombospondin (TSP) is a matrix glycoprotein released from the α-granules of platelets and activated endothelial cells. The aim of this investigation therefore was to examine circulating plasma levels of TSP and the cellular adhesion molecules E-selectin and intra-cellular adhesion molecule in patients with acute coronary syndromes. Thirty four patients with ACS and 24 patients with non-cardiac chest pain were studied. TSP was measured by in-house radioimmunoassay whilst ELAM and ICAM were measured by ELISA methodology. The results are shown below (expressed median [range]):

<table>
<thead>
<tr>
<th>g-wave MI</th>
<th>Nq-MI</th>
<th>Unstable angina</th>
<th>Non card CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>51 (20-80)</td>
<td>51 (27-92)</td>
<td>51 (47-83)</td>
</tr>
<tr>
<td>TSP (ng/ml)</td>
<td>543</td>
<td>2415</td>
<td>804</td>
</tr>
<tr>
<td>ICAM (ng/ml)</td>
<td>1630</td>
<td>1640</td>
<td>1600</td>
</tr>
<tr>
<td>ELAM (ng/ml)</td>
<td>238 (143-275)</td>
<td>355 (104-232)</td>
<td>108 (68-184)</td>
</tr>
</tbody>
</table>

P<0.05 Kruskal Wallis test.

In conclusion therefore patients with acute coronary syndromes have elevated circulating plasma levels of TSP, ICAM and ELAM probably related to platelet and endothelial activation. As thrombospondin also exerts a potent antiangiogenic effect increased levels could be involved in the progression and/or limitation of the pathogenic mechanisms.

**M6**

THE EFFECT OF INHIBITION OF INTEGRINS αIIb3, αVβ3, OR BOTH ON THE ARTERIAL RESPONSE FOLLOWING CORONARY ANGIOPLASTY IN A PORCINE MODEL

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The integrins αIIb3 and αVβ3 play important roles in thrombosis (αIIb3), cell proliferation and migration (αVβ3), processes thought to be involved in restenosis following coronary intervention. We assessed the effect of a selective αIIb3 antagonist (Laminab), a selective αVβ3 antagonist (VO514) and a combined αIIb3/αVβ3 antagonist (G3580) upon porcine vascular smooth muscle cell (VSMC) adhesion and the response to balloon injury in a porcine model.

**Methods:** The effect of each antagonist on VSMC adhesion to vitronectin, fibronectin, laminin and collagen type IV coated wells was assessed using standard methods. For in vivo experiments, 37