4:30

ENDOTHELIUM-DEPENDENT AND -INDEPENDENT cGMP PRODUCTION IS GREATER IN ARTERIAL THAN VENOUS GRAFT TISSUE.

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We have examined the capacity of the human saphenous vein (SV), internal mammary artery (IMA) and gastroepiploic artery (GEA) to generate cyclic GMP, the second messenger that translates EDRF release into smooth muscle relaxation. 287 vessel segments of native SV (SV_N), surgically distended SV (SV_D), IMA and GEA were utilised from 28 patients. Following challenge (45 secs.) with substance P (SP), bradykinin (BK), acetylcholine (Ach) or glyceryl trinitrate (GTN), segments were flash-frozen and assayed for cGMP. The data was normalised to pmol/mg protein. Control (basal) levels of cGMP were: SV_N-0.17±0.03; IMA-0.74±0.08; GEA-8.23±3.5. Agonist stimulation produced increases in cGMP which were significantly higher in the IMA than SV_N or SV_D ($p<0.05$): SV_N, SP (10^{-8}M): 0.24±0.05, BK (10^{-6}M): 0.37±0.08, Ach (10^{-6}M): 0.55±0.16 - IMA, SP: 1.23±0.29, BK: 1.3±0.29, Ach: 1.85±0.74. GTN (1ug/ml) caused further cGMP increase: SV_N-0.7±0.2, SV_D-0.32±0.1, IMA-2.7±0.6. Endothelium removal abolished cGMP increase above control, and levels were always lower in distended segments. All measured parameters in the GEA were 10-fold greater than the IMA ($p<0.05$), control: 8.23±3.5, SP: 14.5±4.0, GTN: 43.8± 13.9. These data suggest that the sensitivity of the guanylate cyclase system varies between blood vessels. This variation may reflect differences in endothelial function between arteries and veins, and may contribute to a vessel's inherent protection against constrictor influences and the documented superiority of arterial grafts.

5:00

VASOACTIVE INTESTINAL PEPTIDE INDUCES HUMAN CORONARY ARTERIAL RELAXATION THROUGH A VARIETY OF MECHANISMS

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We have examined the effects of vasoactive intestinal peptide (VIP) on isolated human epicardial coronary arteries in an attempt to elucidate its mode of action and its possible role in regulating coronary artery tone. Experiments were conducted on 43 ring segments of coronary artery taken from 10 patients (7-60 years) undergoing heart transplantation for non-ischaemic heart disease and examined in isolated organ baths. Vessel segments free of atheroma, preconstricted with the thromboxane A2 mimetic U46619, relaxed to VIP (10^{-12}-10^{-7}M) in a dose-dependent manner. The maximum response produced by VIP was 74.2±4.3% (mean±SEM) of maximum relaxation induced by 1ug/ml glyceryl trinitrate. VIP at 10^{-9}M elicited a relaxation of 22.0±10.4%. Following removal of the endothelium, 30 min incubation with 10^{-6}M indomethecin or 10^{-6}M atropine this response was attenuated to 7.0±4.3% ($p>0.05$) , 5.8±5.8% ($p<0.05$) and 5.8±2.8% ($p<0.05$) respectively. However the response of VIP at 10^{-8}-10^{-7}M was unaffected by any of these treatments. VIP action was attenuated over the whole dose range by prior exposure of the tissues to 10^{-7}M cimetadine (H_2-receptor antagonist) and 10^{-7}M mepyramine (H_1-receptor antagonist) . This treatment inhibited the maximum response of VIP to 51.8±9.4% ($p<0.05$). These results suggest that at low concentrations VIP produced an endothelial dependent relaxation which appears to be mediated by prostacyclin, and acetycholine induced release of endothelium derived relaxing factor. VIP may also act either via the release of histamine or at histaminergic receptors. At high concentrations VIP has direct dilator action on coronary arteries. It is concluded that VIP appears to influence coronary artery tone by a variety of mechanisms.

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DESENSITIZATION OF SOLUBLE GUANYLATE CYCLASE DETERMINES VASCULAR TOLERANCE TO NITROGLYCERIN

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Development of vascular tolerance to nitroglycerin (NTG) has been attributed to sulfhydryl (SH) depletion, guanylate cyclase desensitization, or both. Controversy regarding the precise contribution of these mechanisms may be due to variations in experimental design. To further examine the biochemical basis of NTG tolerance, rat aortic rings with intact endothelium were contracted with norepinephrine and relaxed with NTG. The rings were then rapidly recontracted with a variety of agents and re-exposed to NTG. Re-exposure to NTG resulted in development of tolerance with a 130-fold rightward shift in the NTG dose-response curve (EC_{50} 1.2 x 10^{-5} vs 9.0 x 10^{-8} M, n = 15, $p < 0.01$). However, these NTG-tolerant rings relaxed in response to atrial natriuretic peptide, indicating preservation of particulate guanylate cyclase. Treatment of recontracted rings with the SH-donor N-acetylcysteine (10 μM) did not restore smooth muscle relaxation in response to NTG (EC_{50} 2.0 x 10^{-5} M, n = 8), suggesting that SH depletion may not be the basis of NTG tolerance in these experiments. To examine the role of soluble guanylate cyclase, parallel sets of vascular rings were recontracted with endothelin-1 (n = 5) or the EDRF inhibitor N^G-monomethyl arginine (L-NMMA, n = 8) after initial relaxation with NTG to restore sensitivity of soluble guanylate cyclase. In both endothelin-1 and L-NMMA-treated rings, smooth muscle relaxation in response to NTG was completely preserved (EC_{50} = 7.3 x 10^{-8} M and 8.2 x 10^{-8} M, respectively, p = ns compared with initial NTG exposure). In summary, these data indicate that development of NTG tolerance upon rapid re-exposure of blood vessels to this compound is related to desensitization of soluble guanylate cyclase.

5:15

PERSISTENT ENDOTHELIAL AND ADRENERGIC DYSFUNCTION AFTER ANGIOPLASTY

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We examined the chronic effects of balloon injury on vascular reactivity 3 and 6 weeks after angioplasty (A) of the abdominal aorta or a sham procedure (S) in 21 Sprague-Dawley rats. At 3 weeks two-thirds of the denuded surface was covered with regenerated endothelium and by 6 weeks the endothelium had completely regenerated, although the endothelial cells were morphologically abnormal. Vascular rings of the aorta were studied *in vitro*; vascular smooth muscle function was assessed by examining relaxation to sodium nitroprusside (SNP) and contraction to potassium chloride (KCL) or norepinephrine (NE). Sensitivity to SNP was not different in vascular rings from A compared to S at 3 or 6 weeks, suggesting that the ability of the smooth muscle to relax was unimpaired. Similarly, sensitivity to KCL was not different from S at 3 or 6 weeks, suggesting that contractile function had recovered. Conversely, at both 3 and 6 weeks sensitivity to norepinephrine was reduced by sevenfold (EC50 [-logM] values of 6.4 ± 0.2 v 7.0 ± 0.2, A v S; $p<0.01$).

Endothelial function was assessed by examining relaxation to the calcium ionophore A23187; this relaxation was attenuated by tenfold at 3 and 6 weeks after injury (EC50 [-logM] values of 5.9 ± 0.2 v 6.7 ± 0.2, A v S; $p<0.01$). In sum, the endothelium regenerating after balloon injury is dysfunctional, and appears to produce less endothelium-derived relaxing factor. Reduced release of this vasodilator, which is also known to inhibit vascular smooth muscle growth, may contribute to myointimal hyperplasia. Moreover, the dysfunctional endothelium may be less capable of metabolizing circulating catecholamines, thus exposing the underlying smooth muscle to plasma catechols, which might explain the observed down-regulation of adrenergic response.