P55

Inhibition of Aminopeptidase P Potentiates the Vasodepressor Response to Bradykinin.
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ACE is an important kininase, and recent data suggest that some of the effects of ACE inhibitors (ACEi) are kinin-mediated. Another peptidase which may be involved in kinin metabolism is aminopeptidase P (Am P). We examined whether inhibition of Am P with apstatin (Aps) alters mean blood pressure (BP) responses to bradykinin (Bk). Anesthetized rats were divided into 4 groups: 1) vehicle; 2) Aps, 800 μg/kg; 3) lisinopril (Lis), 40 μg/kg; and 4) Aps+Lis. Five minutes after treatment with the inhibitor(s) or vehicle, we studied: 1) the dose response to Bk (50, 100, 200, 400 ng/kg iv bolus) and 2) the BP response to Bk infusion (400 ng/kg/min for 5 min). Aps did not affect BP responses to angiotensin I or acetylcholine. Aps increased the ΔBP response to bolus Bk, i.e., for 200 ng Bk/kg: control -2.3 ± 0.6, Aps -5.7 ± 0.4 mm Hg ($p<0.01$ vs control), Lis -19.0 ± 2.0 and Aps+Lis -27.3 ± 2.8 mm Hg ($p<0.01$ vs Lis). Aps also increased the duration of the response (area under the curve): Lis 0.55 ± 0.06 and Aps+Lis 0.91±0.05 sec ($p<0.01$). In the Bk infusion studies, BP tended to return to baseline with Lis, whereas it remained decreased with Aps+Lis as shown below. (n=11; *$p<0.001$ vs Lis).

Time of Bk infusion (min)	1.0	3.0	5.0
Lis, ΔBP(mm Hg)	-17.6±2.2	-10.9±2.2	-8.7±1.5
Aps+Lis ΔBP(mm Hg)	-30.0±1.9*	-29.6±1.9*	-24.5±2.4*

These data demonstrate that Am P is an important kininase in vivo. Inhibitors of Am P may be a new tool to further increase the contribution of kinins to the effects of ACEi.

P56

Tissue Kallikrein Gene Therapy Reduces Blood Pressure in Spontaneously Hypertensive Rats
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Extensive studies indicate that the tissue kallikrein-kinin system is involved in blood pressure homeostasis. We recently developed transgenic mice expressing human tissue kallikrein. These transgenic mice are hypotensive and their blood pressure can be reversed by the injection of aprotinin (kallikrein inhibitor) or Hoe140 (bradykinin receptor blocker). We further investigated the potential of human tissue kallikrein gene therapy by injecting the kallikrein gene into the skeletal muscle of spontaneously hypertensive rats (SHR). Expression of the human tissue kallikrein gene in SHR was identified by reverse transcription-PCR followed by Southern blot analysis, ELISA and immunohistochemistry. Injection of the human kallikrein gene into SHR caused a reduction of systemic blood pressure one week post-injection and this effect continued for two months. The blood pressure reduction was more than 20 mmHg compared to the controls which were injected with vector DNA alone. The differences are statistically significant indicating that somatic delivery of the human tissue kallikrein gene induces a sustained reduction of systemic blood pressure in SHR. These results raise the possibility of applying kallikrein gene therapy for treating human hypertensive diseases.

P57

Early Blockade of Bradykinin Receptors Increases Blood Pressure in Rats.
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We evaluated if chronic inhibition of B_2-bradykinin receptors by the long-acting antagonist Hoe140 affects blood pressure (BP) of normotensive rats. Neither Hoe140, at 25 nmol/day per kg body weight (BW) i.p. for 4 weeks, nor its vehicle altered systolic BP (plethysmography) of 9 week-old rats on normal (0.3%) or high (2%) sodium intake. In further experiments, pairs of Hoe140-treated rats were mated and their pups were maintained on Hoe140 and normal sodium diet. Controls were given vehicle instead of Hoe140. Rats given Hoe140 when in utero and then during early phases of the life showed greater systolic BP, heart rate (HR) and BW values than controls at 7 weeks as well as at 9 weeks of age (122±3 vs 113±3 mmHg, 444±6 vs 395±8 b/m, 258±7 vs 213±3 g, $p<.01$). These differences remained unaltered when the rats were fed a high sodium diet while still continuing Hoe140 or vehicle for further 3 weeks (121±1 vs 112±2 mmHg, 399±11 vs 362±4 b/m, 349±5 vs 307±6 g, $p<.01$). By contrast, low sodium diet (0.05 %) nullified the difference in systolic BP (116±2 vs 120±2 mmHg in controls, N.S.). Mean BP (direct measurement) was higher in Hoe140-treated rats on high sodium (115±2 vs 99±2 mmHg in controls, $p<.01$). Propranolol (2 mg/kg, i.v. bolus) induced a greater bradycardic effect in Hoe140-treated rats on high sodium than in controls (105±8 vs 61±10 b/m, $p<.05$), without affecting mean BP. Heart weight was greater in Hoe140-treated rats than in controls (3.00±0.07 vs 2.75±0.04 g/kg BW, $p<.05$). In conclusion, early blockade of bradykinin receptors increases BP and HR, while inhibition in adult life is ineffective. Our data suggest that endogenous kinins may play a role in the regulation of cardiovascular function by influencing the activity of sympathetic nervous system and/or renal excretory function in early phases of life.

P58

Bradykinin Induces Nitric Oxide Production by Rat Carotid Smooth Muscle Cells *in vitro*.
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Bradykinin (BK) and nitric oxide (NO) may mediate part of the protective effect of angiotensin-converting enzyme inhibitors (ACEi). Some of these effects cannot be explained by BK-induced formation of NO by endothelial cells. We hypothesized that BK can increase NO generation by vascular smooth muscle cells (VSMC). Carotid VSMC from Sprague-Dawley rats were plated (passages 4 to 8); 24 hr later, fresh medium, alone or containing either BK (10^{-6} M), ramiprilat (R, 10 μg/ml) or BK+R, was added. At 5 and 20 hr after treatment, nitrite (NO_2^-) was measured in the medium. Treatment for 5 hr resulted in no detectable increase in NO_2^-, while in cells treated 20 hr with BK+R NO_2^- production ($n = 23$) increased by 213% (from 3.1±0.7 to 6.7 ± 1.0 nmol/mg protein, $p < 0.004$). In dose response studies BK at 10^{-8} M, plus R, still induced significant increases in NO_2^- (Control, 0.11± 0.02; 10^{-6} M BK, 3.18±0.11; 10^{-7} M BK, 3.02±0.30; 10^{-8} M BK, 1.16±0.09 [$n = 3$, $p < 0.0001$]). BK or R alone did not induce changes in NO_2^-. In contrast, BK alone increased the production of prostacyclin and PGE_2 (6-keto $PGF_{1\alpha}$: Control= 1.4±0.2, BK= 23.3±3.5, BK+R= 29.5±4.1 ng/mg protein [$n = 4$, $p < 0.001$]; PGE_2: Control= 7.0±1.4, BK= 47.2±19.8, BK+R= 44.2±12.3 [$n = 4$, $p < 0.03$]). Treatment with dexamethasone (10^{-5} M), which inhibits expression of inducible nitric oxide synthase (iNOs), or with the NO synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (840 μg/ml), abolished responses to BK+R ($n = 3$).. The increase in NO_2^- and prostanoids was completely blocked by Hoe 140 (10^{-4} M), a BK B_2 receptor antagonist. We conclude that stimulation of BK B_2 receptors induces NO generation in VSMC, likely by increasing iNOs. Inhibition of BK degradation by ACE is needed to reveal the effects of BK on NO formation. The present data suggest that in addition to the known ability of kinins to release factors such as NO, prostacyclins and hyperpolarizing factor from endothelial cells, in the presence of an ACEi kinins can also increase production of vasodilatory and anti-mitogenic factors from VSMC.

P59

Intramuscular Delivery of Rat Kallikrein-binding Protein Gene Reverses Hypotension in Transgenic Mice Expressing Human Tissue Kallikrein
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The tissue kallikrein-kinin system has been postulated to play an important role in blood pressure regulation. Rat kallikrein-binding protein (RKBP) is a serine proteinase inhibitor which binds to and inhibits tissue kallikrein's activity. We recently developed several lines of human tissue kallikrein transgenic mice which are hypotensive. In order to investigate the role of RKBP in blood pressure regulation, we delivered the RKBP gene into these transgenic mice by intramuscular injection. Expression of the RKBP gene was detected in the skeletal muscle by reverse transcription-PCR followed by Southern blot analysis at 10, 20, 30 and 40 days post-injection. Immunoreactive RKBP levels in the muscle and serum of these mice were quantified by a RKBP-specific ELISA and Western blot analysis. RKBP mRNA and protein levels were low at 10 days post-injection but increased significantly at 20 and 30 days. During this time period, RKBP gene delivery significantly increased systemic blood pressure in the kallikrein transgenic mice to a level comparable to that of normotensive control mice. Neither the RKBP gene nor vector DNA delivery had an effect on the blood pressure of normotensive control mice. These results suggest that the increase of systemic blood pressure by RKBP gene delivery in these hypotensive transgenic mice might be mediated by inhibiting tissue kallikrein activity.

P60

Blockade of ANG II (Subtype AT_1) Receptors induces Kinin and Prostacyclin Release from isolated perfused Rat Hearts
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We have recently shown that reduction of postischemic reperfusion arrhythmias in isolated rat hearts by AT_1 receptor antagonists seems to be mediated via endogenous ANG II, probably through stimulation of cardiac endothelial AT_2 receptors. Since this cardioprotective effect is prevented by coperfusion with the B_2-kinin receptor antagonist icatibant we investigated the possible contribution of cardiac kininogenase-kinin pathways during AT_1 receptor blockade. In normoxic with Krebs-Henseleit buffer perfused hearts AT_1 receptor antagonists significantly enhanced the outflow of kinins into the venous effluent (from 166.4±32 to 518.0±42 fmol/min). In parallel an increased prostacyclin outflow was observed. In ischemic hearts the decrease in reperfusion arrhythmias by AT_1 receptor antagonists was suppressed by coperfusion with either the serine or the cysteine protease inhibitors dichloroisocoumarin and E 64 respectively. The data suggest that the cardioprotective action by AT_1 receptor blockade is accompanied by an enhanced synthesis and release of endogenous kinins. By promoting NO and prostacyclin synthesis kinins are suggested to protect isolated rat hearts against endothelial dysfunction and myocardial ischemia.