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Inhibition of Aminopeptidase P Potentiates the Vasodpressor Response to Bradykinin. Shin-ichi Kitamura, Luis A. Carbin, Oscar A. Carretero, William H. Simmons*, A. Guillermo Scichi. Henry Ford Hospital, Detroit, MI and *Loyola Univ., Maywood, IL

ACE is an important kininase, and recent data suggest that some of the effects of ACE inhibitors (ACEI) are kinin-mediated. Another peptide which may be involved in kinin metabolism is aminopeptidase P (AP). We examined whether inhibition of AP with aprotinin (Ap) alters mean blood pressure (BP) responses to bradykinin (Bk). Anesthetized rats were divided into four groups: 1) vehicle; 2) Ap, 800 

μg/kg; 3) lisinopril (Lis), 40 μg/kg; and 4) Ap+Lis. Five minutes after treatment with the inhibitor(s) or vehicle, we studied: 1) the dose response to Bk (50, 100, 200, 400 ng/ml in buffer) and 2) the BP response to Bk infusion (400 ng/kg/min for 5 min). Ap did not affect BP responses to angiotensin I or acetylcholine. Ap increased the Bk response to both Bk, i.e., for 200 ng Bk: control = 2.± 0.6, Ap -5.± 0.4 mm Hg (p<0.01 vs control), Lis = 19.± 2.0 and Ap+Lis = 27.3± 2.8 mm Hg (p<0.01 vs Lis). Also ap isolated the duration of the response (area under the curve). Lis 0.55± 0.06 and Ap+Lis 0.19±0.05 sec (p<0.01). In the BP infux studies, Bk tended to return to baseline, whereas it remained decreased with Ap+Lis as shown below. (n=11; *p<0.001 vs Lis).

Time of Bk infux (min)

1.0 2.0 3.0 5.0

Lis, Ap+Bk (mm Hg) -7.6±2.2 -10.9±2.2 -8.7±1.5

Ap+Bk (Ap+Bk Hg) -30.0±2.0* -29.6±2.0* -24.5±2.4*.

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

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Tissue Kallikrein Gene Therapy Reduces Blood Pressure in Spontaneously Hypertensive Rats. Lee Chao, William Xiong, Julie Chao. Medical University of South Carolina, Charleston, SC

Extensive studies indicate that the tissue kallikrein-kinin system is involved in blood pressure homeostasis. We recently developed transgenic mice expressing human tissue kallikrein. The 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 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 mm Hg compared to the controls which were injected with vector DNA alone. The differences are statistically significant indicating that somatic delivery of the human kallikrein gene induces a sustained reduction of systemic blood pressure in SHR. These results raise the possibility of applying kallikrein gene therapy for treating hypertensive diseases.

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Early Blockade of Bradykinin Receptors Increases Blood Pressure in Rats. Paolo Madeddu, Paola Pinni Parrapage, Maria Vittoria Varoni, Nicola Glorioso. Clinica Medica e Farmacologia, University of Sassari, Sassari, Italy

We evaluated if chronic inhibition of B1-bradykinin receptors by the long-acting antagonist Hoe140 affects blood pressure (BP) of normotensive rats. Neither Hoe140 at 23 mmol/day per kg body weight (BW) i.p. for 4 weeks, nor its vehicle altered systolic BP (plithysmography) 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 life showed lower 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 mmHg, 258±7 vs 237±5 g, p<0.01). These differences remained unaltered if 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 mmHg, 345±9 vs 307±6 g, p<0.01). 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<0.01). Propranolol (2 mg/kg, i.v.) injected a bradykinin antagonist (B1) the activity of sympathetic nervous system and/or renal excretory function in early phases of life.

Bradykinin Induces Nitric Oxide Production by Rat Cardiot Smooth Muscle Cells as roor. Luis A. Carbin and A. Guillermo Scichi. Henry Ford Hospital, Detroit, MI, USA

Bradykinin (BK) and nitric oxide (NO) may mediate part of the protective effect of angiotensin-converting enzyme inhibitors (ACE). 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). Cardiot VSMC from Sprague-Dawley rats were plated (passages 4 to 8), 24 hr later, fresh medium, alone or containing ether BK (10*M), n-pentane (R, 10 μg/ml) or BK+R, was added. At 5 and 20 hr after treatment, nitrite (NO2) was measured in the medium. Treatment for 5 hr resulted in no detectable increase in NO2; while in cells treated 20 hr with BK+R, NO2 production (n = 23) increased by 213% (from 3.1±0.7 to 6.7±1.0 nmol/mg protein, p<0.004). In other experiments, we studied BK at 10*M, plus ACEI (baxagliptin, Candesartan, and NO), or the BK antagonist Hoe 140. (Control, 0.11±0.02; 10*M BK, 3.18±0.11; 10*M BK, 3.02±0.30; 10*M BK, 1.16±0.09 (p = 3.8, p<0.001)). BK or R alone did not induce changes in NO2. In contrast, BK increased the production of prostanoid and PGE2 (keto PGE2, p<0.02). BK: 2.47±0.8; BK+R: 2.52±0.3; BK+R (n = 4, p<0.001). Treatment with desamethasone (10−6 M), which inhibits expression of inducible nitric oxide synthase (iNOS), or with the NO synthase inhibitor N-nitro-l-arginine methyl ester (840 μM), abolished responses to BK+R (n = 3). The increase in NO2 and prostanoids was completely blocked by Hoe 140 (10*M), a BK receptor antagonist. We conclude that stimulation of BK B2 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 ACE inhibitors can also increase production of vasodilatory and anti-mitogenic factors from VSMC.

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Intramuscular Delivery of Rat Kallikrein-binding Protein Gene Reverses Hypertension in Transgenic Mice Expressing Human Tissue Kallikrein. Julie Chao, Jian-xing Ma, Lee Chao. Medical University of South Carolina, Charleston, SC

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 protease 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 RBKBP in the 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 RBKBP levels in the muscle and serum of these mice were quantified by a RKBP-specific ELISA and Western blot analysis. RBKBP mRNA and protein levels were low at 10 days post-injection but increased significantly at 20 and 30 days. During this time period, RBKBP 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 RBKBP gene delivery in these hypotensive transgenic mice may be mediated by inhibiting tissue kallikrein activity.

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Blockade of Ang II (Subtype A1β) Receptors induces Kinin and Prostacyclin Release from Isolated perfused Rat Hearts. Gabriele Niemer, Edwin Flank, Petra Korth, Paulus Wohlfart, Bernward A. Schölkens, Wolfgang Linz. Hoescht AG, Frankfurt/M, Germany, University München, Germany

We have recently shown that reduction of postischemic reperfusion arrhythmias in isolated rat hearts by A1 receptor antagonists seems to be mediated via endogenous Ang II, probably through stimulation of cardiac endothelial AT1 receptors. Since this cardioprotective effect is prevented by coperfusion with the BK-kinin receptor antagonist icatibant we investigated the possible contribution of cardiac BK receptors to postischemic arrhythmias during A1 receptor blockade. We perfused normoxic with Krebs-Henseleit buffer perfused hearts AT1 receptor antagonists significantly enhanced the outflow of kinins into the venous effluent (from 16.4±32 to 518.0±42 femtomol/min). In contrast, an increased outflow was observed in ischemic hearts. These differences in reperfusion arrhythmias by AT1 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 of AT1 receptor blockade is accompanied by an enhanced synthesis and release of endogenous kinins. By promoting NO and prostacyclin synthesis kinins are suggested to promote isolated rat hearts against endothelial dysfunction and myocardial ischemia.