EFFECT OF INSULIN ON BRADYKININ-INDUCED INTRACELLULAR CALCIUM MOBILIZATION IN CULTURED HUMAN ENDOTHELIAL CELLS.

Y. Takagawa, MT Hori, and ML Tuck. Veterans Administration GLHCS, Sepulveda, CA and UCLA School of Medicine, Los Angeles, CA

The acute vasodilatory effects of insulin are now generally established and it is also possible that insulin resistance reduces this vasodilatory effect. The precise mechanism(s) of insulin-induced vasoconstriction are only partially understood. One possible pathway of insulin-mediated endothelial cell dysfunction may be nitric oxide synthase inhibition via elevation of intracellular calcium ([Ca²⁺]) in endothelial cells. To further elucidate insulin's effect on this mechanism, we investigated the effect of insulin on bradykinin (BK) induced [Ca²⁺]i responses in cultured human arterial vascular endothelial cells (HAVEC) using the calcium-indicating dye Fluo-3 AM. The effects of insulin (100 µM/ml) on basal and peak BK-induced [Ca²⁺]i ([Ca²⁺]) (10 µM), as well as on the sustained [Ca²⁺]i response 10 minutes after BK addition were compared. Inulin treatment had no effect on peak calcium levels. Peak BK-induced intracellular calcium was elevated by insulin preincubation compared to vehicle control (152.9±16.7 vs. 147.4±22.2 nM, respectively). Moreover, 10 minutes after BK stimulation [Ca²⁺]+ remained elevated in the insulin treated group (126.0±12.2 nM), while the control group returned more rapidly towards basal levels (109.9±17.2 nM, p <0.05). We conclude that insulin may affect BK-induced vasodilatory responses by modulating calcium signaling pathways in HAVEC.

Key Words: Vascular endothelium, calcium, bradykinin, insulin

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CYCLOOXYGENASE-2 (COX-2) IN THE THICK ASCENDING LIMB: REGULATION AND FUNCTION.


Tumor necrosis factor-α (TNF) acts in an autocrine manner to inhibit Na⁺ uptake in the medullary thick ascending limb (mTAL). This effect, which is consistent with the observed natriuretic action of TNF, occurs via a prosaposin-dependent mechanism that requires induction of COX-2 gene transcription and protein expression. Pretreatment of mTAL cells with dexamethasone (DEX; 10 µM) inhibited TNF-mediated COX-2 expression by approximately 95%. Thus, we assessed the effects of adrenocorticotropic hormone (Adx) on renal COX-2 expression. COX-2 expression was evident in a subset of tubular epithelial cells located in the cortex and outer medulla. Staining of serial sections showed that COX-2-positive cells also contained Tamm-Horsfall glycoprotein, a highly selective marker for TAL cells. These cells were located at a considerable distance from the corresponding macula densa, although they occasionally were close to glomeruli. Less than 2% of TAL cells were COX-2-positive in sham-operated rats, whereas more than 25% of TAL cells expressed COX-2 after Adx. Treatment of Adx rats with DEX (Adx-Dx, 1 mg/kg) reduced the number of COX-2-positive cells to levels observed in sham-operated rats (1-2%). Western blot analysis of cortical microsomes revealed a COX-2 protein band in Adx rats but not in Adx-Dx or sham-operated rats. Similarly, COX-2 mRNA accumulation was greatly increased by Adx and was completely abolished by treatment with DEX. The increase in COX-2 mass observed after Adx was associated with expression of active enzyme as demonstrated by a 3-fold increase in PGE₂ formation by cultured TAL cells. The increase in COX-2 expression of enzymatically active COX-2 in the TAL, and 2) glucocorticoids act as endogenous inhibitors of COX-2 in the TAL.

Key Words: Cyclooxygenase-2, TNF, kidney, glucocorticoids, prostanoids

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The ability of the D₁-like receptor to stimulate adenyl cyclase (AC) and phospholipase C (PLC), inhibit sodium transport in the renal proximal tubule (RPT), and produce natriuresis is attenuated in several rat models of hypertension. Since the inhibitory effect of D₁-like receptors on RPT sodium transport is also reduced in some patients with essential hypertension, we measured D₁-like receptor coupling to AC in cultures of human RPT cells from normotensive (NT) and hypertensive (HT) subjects. Basal CAMP concentrations were the same in NT (n=7) and HT (n=5) tissues. However, D₁-like receptor agonist, fenoldopam, increased cAMP production to a greater extent in NT (maximum response [Emax] = 67 ± 1%) than in HT (Emax = 17 ± 5 %). Dopamine also increased CAMP production to a greater extent in NT (32 ± 3%) than in HT (14 ± 3 %). The fenoldopam-mediated increase in cAMP production was blocked by SCH 23390 (a D₁-like receptor antagonist) and by antiserum against D₁ oligonucleotides in both HT and NT, indicating action at the D₁ receptor. The stimulatory effects of forskolin and parathyroid hormone-related protein (PTHrP) of cAMP accumulation were not statistically different in NT and HT, indicating receptor specificity and an intact G-protein/AC pathway. The fenoldopam-stimulated PLC activity was not impaired in HT, and the primary sequence and functional studies of the D₁ receptor were the same in NT and HT. However, D₁ receptor sensitivity phosphorylation in the basal state was greater in HT (n=3) than in NT (n=4) and was not responsive to fenoldopam stimulation in HT. We conclude that uncoupling of the D₁ receptor in renal proximal tubules in both rats and humans may be due to ligand-independent phosphorylation of the D₁ receptor in hypertension. Gene or genes involved in the phosphorylation of the D₁ receptor may be important in the pathogenesis of hypertension.

Key words: D₁ receptor, adenyl cyclase, phospholipase C, serine phosphorylation

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Omapatrilat, a novel vasopeptidase inhibitor, is a single molecule that simultaneously inhibits neutral endopeptidase (NEP) and angiotensin converting enzyme (ACE). Inhibition of NEP protects vasodilator peptides, inhibits renin/angiotensin II and aldosterone production, decreases blood pressure in low renin states. ACE inhibition attenuates the D₁-like receptor coupling to AC in proximal tubules. The purpose of this study was to determine if omapatrilat produces dose-related antihypertensive efficacy in models of hypertension in both renin states. The study was conducted in normotensive (NT) and hypertensive (HT) spontaneously hypertensive rats (SHR, normal renin) and deoxycorticosterone acetate-salt hypertensive rats (DOCA, low renin) were treated with oral omapatrilat at 0, 10, 36, or 100 µmol/kg once daily for 7 days. Baseline systolic blood pressure (SBP) in SHR before each dose was 199±4, 188±3, 198±4, and 218±5 mm Hg. Results are summarized in the table. Plasma and lung ACE activity was decreased 24 h after the last dose in all treatment groups (P<0.05). These results indicate that Omapatrilat produces dose-related antihypertensive efficacy in both normal and low renin experimental hypertension and predicts clinical antihypertensive efficacy, independent of renin status.

Key Words: Omapatrilat, vasopeptidase inhibitor, experimental hypertension, DOCA, SHR

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Maximum change in SBP (%)

<table>
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<th>Vehicle</th>
<th>10</th>
<th>30</th>
<th>100</th>
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</thead>
<tbody>
<tr>
<td>SHR</td>
<td>-8±2</td>
<td>9±3</td>
<td>14±3</td>
</tr>
<tr>
<td>DOCA</td>
<td>-8±2</td>
<td>-23±6</td>
<td>-15±6</td>
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</tbody>
</table>

Plasma and lung ACE activity was decreased 24 h after the last dose in all treatment groups (P<0.05). These results indicate that Oma produces dose-related antihypertensive efficacy in models of hypertension in both renin states. The study was conducted in normotensive (NT) and hypertensive (HT) spontaneously hypertensive rats (SHR, normal renin) and deoxycorticosterone acetate-salt hypertensive rats (DOCA, low renin) were treated with oral omapatrilat at 0, 10, 36, or 100 µmol/kg once daily for 7 days. Baseline systolic blood pressure (SBP) in SHR before each dose was 199±4, 188±3, 198±4, and 218±5 mm Hg. Results are summarized in the table. Plasma and lung ACE activity was decreased 24 h after the last dose in all treatment groups (P<0.05). These results indicate that Omapatrilat produces dose-related antihypertensive efficacy in both normal and low renin experimental hypertension and predicts clinical antihypertensive efficacy, independent of renin status.

Key Words: Omapatrilat, vasopeptidase inhibitor, experimental hypertension, DOCA, SHR