Effect of Insulin, Proinsulin, and Amylin on Renin Release From Perfused Rat Kidney

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To evaluate the possible role of insulin, proinsulin, and amylin in the renin-angiotensin system, the direct effect of these peptides on renin release was examined using perfused kidney of rats. Renin release was significantly increased from a basal value of 6.1 ± 1.8 to a peak value of 10.1 ± 2.3 ng angiotensin I (Ang I)/mL/h by 0.5 nmol insulin, from 6.0 ± 1.7 to 16.7 ± 4.5 ng Ang I/mL/h by 1 nmol insulin, and from 6.1 ± 1.8 to 18.0 ± 5.5 ng Ang I/mL/h by 8 nmol insulin. Renin release was not significantly changed by perfusion of 0.05 nmol proinsulin or amylin but significantly increased from a basal value of 6.1 ± 1.7 to a peak value of 8.1 ± 3.6 ng Ang I/mL/h by 1 nmol proinsulin, from 5.6 ± 1.7 to 12.1 ± 3.8 ng Ang I/mL/h by 8 nmol proinsulin, from 5.7 ± 1.9 to 8.2 ± 3.5 ng Ang I/mL/h by 1 nmol amylin, and from 5.2 ± 2.0 to 12.4 ± 3.3 ng Ang I/mL/h by 8 nmol amylin. The concentration of cyclic adenosine monophosphate in the effluent was significantly increased from a basal value of 5.1 ± 1.6 to a peak value of 10.6 ± 2.5 mmol/min by 8 nmol amylin but not altered by perfusion of insulin or proinsulin. The addition of 0.05 nmol proinsulin and 0.05 nmol of amylin on 0.5 nmol insulin significantly enhanced renin release. These results indicate that insulin may play an important physiologic role in the renin-angiotensin system and suggest that proinsulin and amylin may be involved in the genesis and development of hypertension through enhancement of insulin-stimulated renin release.

INSULIN RESISTANCE and hypertension are commonly associated. However, the underlying cause of this association is unknown. Hyperinsulinemia, hyperproinsulinemia,[1,2] or hyperamylinemia[3,4] have been reported in insulin resistance. Insulin resistance is reported to be associated with elevated levels of plasma renin activity in patients with untreated essential hypertension.[5] Insulin is known to stimulate renin release in humans,[6,7] dogs,[8] and rats[9] in vivo, probably because of subsequent hypoglycemia. Two reports of in vitro experiments suggest that insulin directly stimulates renin release in rat renal cortical slices[10] and perfused guinea pig kidney,[11] and 1 study reached a different conclusion in the perfused rat kidney.[12]

The risk factors associated with the metabolic syndrome, such as low high-density lipoprotein (HDL) cholesterol, high triglyceride levels, high arterial blood pressure, increased waist-hip ratio, and plasminogen activator inhibitor levels are better correlated with proinsulin and split proinsulin levels than with the level of insulin itself.[13,14] However, the possible role of proinsulin for hypertension remains to be elucidated.

Plasma concentrations of amylin, which is cosecreted with insulin by the pancreatic β cell, are reported to be elevated in various states of insulin resistance, including essential hypertension,[15] and it has recently been suggested that amylin-induced activation of renin may be an important mechanism in the association of hypertension with hyperamylinemic states such as the insulin resistance syndrome.[3,4] However, the direct effect of amylin on renin release is unknown.

To elucidate these issues, in the present study, direct effects of insulin, proinsulin, or amylin on renin release were examined using perfused rat kidneys.

MATERIALS AND METHODS

Animals

Studies were carried out in male Wistar rats weighing 200 to 250 g (Japan SLC, Hamamatsu, Japan). The rats were kept for 1 week in an air-conditioned room at 22 ± 2°C with a 12-hour light-dark cycle and were allowed free access to laboratory rat chow and tap water.

Materials

Dextran T-70 was purchased from Green Cross Co, Osaka, Japan. Bovine serum albumin (BSA; (fraction V) were purchased from Sigma Chemical Co, St Louis, MO. Rat amylin was purchased from Peptide Institute, Inc, Osaka, Japan. Human proinsulin was obtained from Sigma Chemical Co. Human insulin was obtained from Novo, Copenhagen, Denmark.

Perfusion of the Kidney

The technique for preparation of the isolated, perfused rat kidney was a modification[16] of the method of Nishiitsutsuji-Uwo et al.[17] The fed rats were anesthetized with intraperitoneal pentobarbital sodium (30 mg/kg), and the abdomen was opened. Loose ligatures were placed around the inferior vena cava above and below the right renal vein and the right renal artery. The polyethylene cannula (1F scale) was inserted into the right ureter to collect the urine. An inflow cannula was inserted through the superior mesenteric artery into the right renal artery. The ligature around the vena cava above the right renal vein was tied, and an outflow cannula was inserted into the vena cava. Finally, the ligature around the vena cava below the right renal vein was tied. The kidney was then isolated and perfused without recirculation with a synthetic medium at a flow rate of 4.0 mL/min.

Perfusion Medium and Perfusion Method

The basal perfusion medium consisted of a Krebs-Ringer bicarbonate buffer containing 0.5% BSA, 4.6% Dextran T-70, and 5.5 mmol/L glucose. Insulin, proinsulin, or amylin was dissolved in the perfusion medium immediately before the perfusion study. The control kidney was perfused for 40 minutes with the basal perfusion medium. The experimental kidney was perfused for 20 minutes with the basal perfusion medium. The kidney was then perfused with the medium containing insulin (0.5, 1, or 8 nmol), proinsulin (0.05, 1, or 8 nmol), amylin (0.05, 1, or 8 nmol), or 0.5 nmol insulin + 0.05 nmol proinsulin + 0.05 nmol amylin, and changed to the basal perfusing medium for 10 minutes. The venous effluent was collected every minute and

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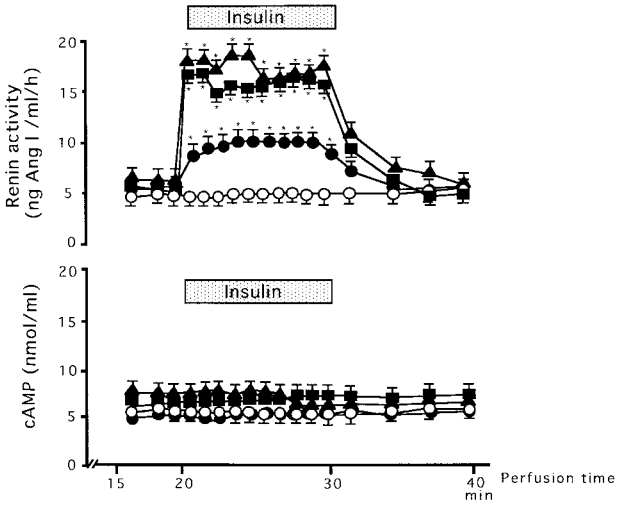

Fig 1. Effect of insulin on renin release. Bars represent means ± SD. (○), control (n = 6); (●), 0.5 nmol insulin (n = 6); (■), 1 nmol insulin (n = 6); (▲), 8 nmol insulin (n = 6). *P < .001, significantly different from control.

stored at −70°C until the time of assay. During perfusion, the medium and the perfusion chamber were warmed and kept at 37°C, and the medium was bubbled with a mixture of 95% O_2 and 5% CO_2. The pH was maintained at 7.4.

Measurements of Renin Activity and Cyclic Adenosine Monophosphate

Venous effluent (100 μL) was incubated with 500 ng of rat renin substrate (Bachem Co, Torrance, CA) for 1 hour to produce angiotensin I (Ang I). Ang I was measured by radioimmunoassay using the double antibody method, and renin activity was calculated. The intraassay and interassay coefficients of variation in renin assay were 5% and 8%. The least detectable concentration was 0.2 ng Ang I/mL/h. Dibutyryl cyclic adenosine monophosphate (cAMP) concentration in the effluent was measured by radioimmunoassay.

Calculation

Oxygen consumption in the kidney was calculated from the difference between influent and effluent oxygen concentrations. The incremental area in renin release for 10 minutes after stimulation (ΣΔ renin activity, ng/Ang I/mL) was calculated by as the sum of increments in renin activity after stimulation at all time points.

Statistical Analysis

Analysis of variance and 2-tailed Student nonpaired *t* test were used for statistical analysis.

RESULTS

Perfusion Pressure, Oxygen Consumption, and Urine Volume

The perfusion pressure was 85 to 95 mm Hg initially, and then it gradually increased to 95 to 105 mm Hg throughout the perfusion. The oxygen consumption was 0.4 to 0.5 μmol O_2/min/g. Urine volume was 800 to 1,000 μL/30 min. Perfusion pressure, perfusate flow rate, oxygen consumption, and urine volume were not changed by perfusion of insulin, proinsulin, or amylin.

Effect of Insulin on Renin Release

As shown in Fig 1, renin activity significantly increased from a basal value of 6.1 ± 1.8 to a peak value of 10.1 ± 2.3 ng Ang I/mL/h by perfusion of 0.5 nmol insulin, from 6.0 ± 1.7 to 16.7 ± 4.5 ng Ang I/mL/h by 1 nmol insulin, and from 6.1 ± 1.8 to 18.0 ± 5.5 ng Ang I/mL/h by 8 nmol insulin. The concentration of cAMP in the effluent was not altered by the perfusion of insulin.

Effect of Proinsulin on Renin Release

As shown in Fig 2, renin activity did not significantly change by perfusion of 0.05 or 1 nmol proinsulin. Renin activity was significantly increased from a basal value of 5.6 ± 1.7 to a peak value of 12.1 ± 3.8 ng Ang I/mL/h by perfusion of 8 nmol proinsulin. The concentration of cAMP in the effluent was not altered by perfusion of proinsulin.

Effect of Amylin on Renin Release

As shown in Fig 3, renin activity did not significantly change with perfusion of 0.05 nmol amylin. Renin activity was slightly but not significantly increased by perfusion of 1 nmol amylin, and that was significantly increased from a basal value of 5.2 ± 2.0 to a peak value of 12.4 ± 3.3 ng Ang I/mL/h by perfusion of 8 nmol amylin. The concentration of cAMP in the effluent was significantly increased from a basal value of 5.1 ± 1.6 to a peak value of 10.6 ± 2.5 nmol/min by perfusion of 8 nmol amylin.

Incremental Area in Renin Release (Σ Δ Renin Activity)

As shown in Fig 4, the Σ Δ renin activity was 26.4 ± 11.2 ng Ang I/mL/h in the kidney perfused with 0.05 nmol insulin, 78.4 ± 24.3 ng Ang I/mL/h with 1 nmol insulin, and 90.6 ± 31.5 ng Ang I/mL/h with 8 nmol insulin. The Σ Δ renin activity was 15.8 ± 4.9 ng Ang I/mL/h with 1 nmol amylin, 62.1 ± 18.3 ng Ang I/mL/h with 8 nmol amylin, 7.4 ± 2.8 ng Ang I/mL/h with 1 nmol proinsulin, and 44.4 ± 15.0 ng Ang I/mL/h with 8 nmol proinsulin.

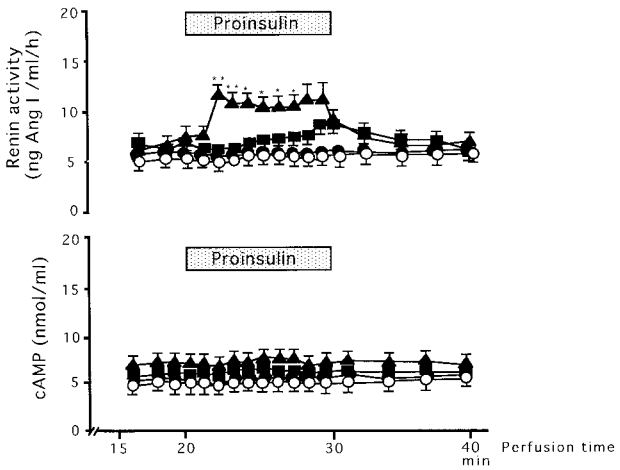

Fig 2. Effect of proinsulin on renin release. Bars represent means ± SD. (○), control (n = 6); (●), 0.05 nmol proinsulin (n = 6); (■), 1 nmol proinsulin (n = 6); (▲), 8 nmol proinsulin (n = 6). *P < .05 and **P < .02, significantly different from control.

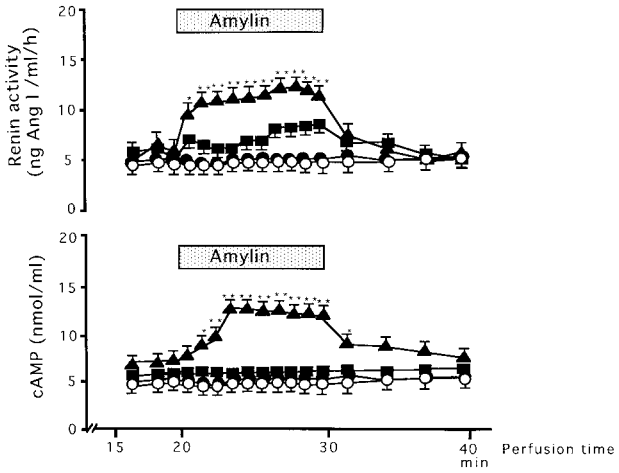

Fig 3. Effect of amylin on renin release. Bars represent means ± SD. (○), control (n = 6); (●), 0.05 nmol amylin (n = 6); (■), 1 nmol amylin (n = 6); (▲), 8 nmol amylin (n = 6). *$P < .05$ and **$P < .02$, significantly different from control.

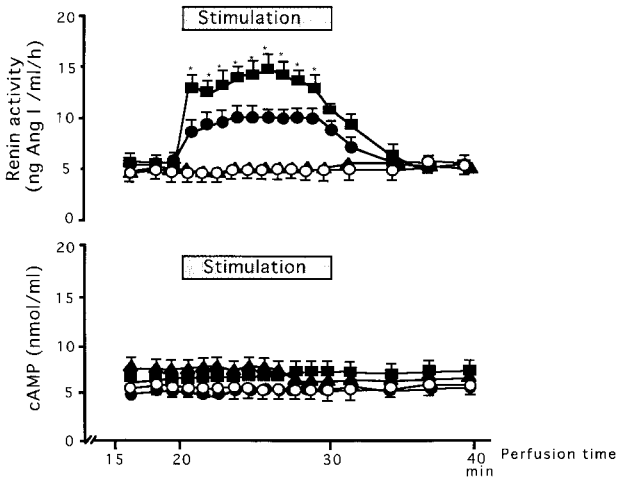

Fig 5. Combined effect of insulin, proinsulin, and amylin on renin release Bars represent means ± SD. (○), 0.05 nmol amylin (n = 6); (▲), 0.05 nmol proinsulin (n = 6); (●), 0.5 nmol insulin (n = 6); (■), combination of amylin, proinsulin, and insulin (n = 6). *$P < .05$, significantly different from 0.5 nmol insulin.

Combined Effect of Insulin, Proinsulin, and Amylin on Renin Release

As shown in Fig 5, renin activity was increased from a basal value of 5.1 ± 1.9 to a peak value of 14.2 ± 3.2 ng Ang I/mL/h by combined perfusion with 0.5 nmol insulin, 0.05 nmol proinsulin, and 0.05 nmol amylin. Renin activity was significantly higher than that with 0.5 nmol insulin. The concentration of cAMP in the effluent was not altered by combined perfusion of physiologic insulin, proinsulin, and amylin.

DISCUSSION

Three pancreatic peptides (insulin, proinsulin, and amylin) in nanomole concentrations used in the present study had direct stimulating effects on renin release from perfused rat kidney. Among these peptides, insulin had the strongest stimulating effect on renin release. Namely, insulin stimulated renin release in the physiologic concentration (0.05 or 1 nmol). The present result that insulin has a direct stimulating effect on renin release is compatible with previous in vivo literature in humans,[6,7] dogs,[8] and rats[9] and in vitro study in rat renal cortical slices[10] and perfused guinea pig kidneys,[11] suggesting that insulin directly plays an important physiologic role in the renin-angiotensin system. On the other hand, Cohen et al[12] reached a different conclusion in perfused rat kidney, probably because their animals were on a low-sodium diet, which influences renin release, and Ang II receptor binding[18,19] and/or perfusion flow rate (34 mL/min/g) was very high in their study.

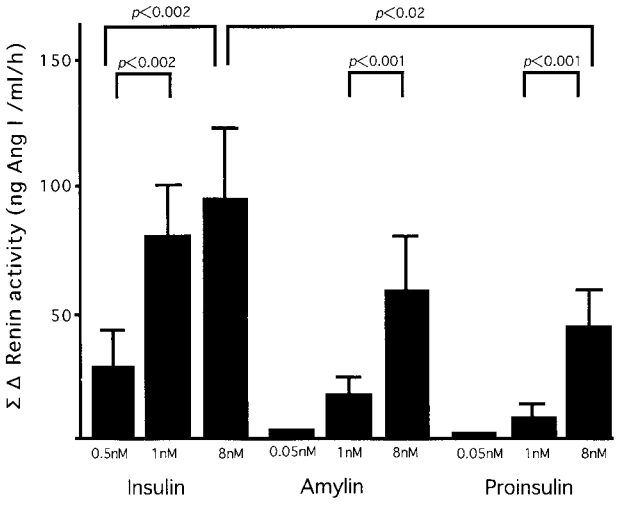

Fig 4. ΣΔRenin activity from perfused kidney. Bars represent means ± SD

To date, there have been no reports regarding the effect of proinsulin on renin release. Proinsulin directly stimulated renin release from the kidney in the present study. However, this effect of proinsulin was not observed in the physiologic concentration (0.05 nmol) but was observed in the supraphysiologic concentration (1 and 8 nmol). Insulin or proinsulin stimulated renin release rapidly. These rapid effects are not caused by mitogenesis and growth per se, suggesting that this is an effect on stored renin or by activation of conversion of inactive to active renin.[20] cAMP is known to be involved in renin release.[21,22] In the present study, insulin and proinsulin did not influence cAMP concentration in the effluent, suggesting that cAMP mechanisms may not be involved in insulin- or proinsulin-stimulated renin release.

Several investigators have reported that amylin stimulates renin release in vivo in humans and rats.[23,24] However, the direct effect of amylin on renin release has not been examined. In the present study, amylin in supraphysiologic concentrations (1 and 8 nmol) had a direct stimulating effect on renin release, although a physiologic concentration (0.05 nmol) of amylin had no significant effect. Wookey et al[24] report that amylin stimulated adenylyl cyclase activity 3- to 4-fold in rat renal cortex. In the present study, 8 nmol amylin increased cAMP concentra-

tion approximately 2-fold in the effluent. Although the effluent cAMP concentration could not correctly show cAMP production in the kidney, the present results indicate that cAMP mechanisms may be involved in amylin-stimulated renin release. Thus, the mechanisms whereby amylin stimulates renin release seem to be different from that of insulin or proinsulin.

An important result obtained in our present study is that the addition of physiologic concentrations of proinsulin (0.05 nmol) and amylin (0.05 nmol) on insulin (0.5 nmol) significantly enhanced renin release, although proinsulin or amylin itself had no significant effect on renin release in physiologic concentrations. These findings indicate that both proinsulin and amylin have important physiologic roles for renin release in the presence of insulin. Although the mechanisms by which insulin directly stimulates renin release are unknown and those by which the addition of proinsulin and amylin to insulin enhances renin release are unclear, in insulin resistance or type 2 diabetes, which frequently associated with hyperinsulinemia, hyperproinsulinemia, and hyperamylinemia, these 3 peptides (insulin, proinsulin, and amylin) may be involved in the genesis and development of hypertension through activation of the renin-angiotensin system. Further studies of the role of proinsulin and amylin in the genesis and development of hypertension in insulin resistance are needed.

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