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Chemiosmotic Control of Renin Secretion

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I. Introduction

Secretion of renin by the juxtaglomerular(JG) cell involves multiple sequential steps including biosynthesis of renin, packaging in the secretory granules, cytoplasmic translocation of the granules close to the plasma membrane, and finally exocytotic release of renin by fusion and then fission of the granule membrane with the juxtaposed plasma membrane.[1] Upon stimulation, only a few percent of the total stored renin is secreted in vivo and in vitro without significant changes in renal renin content.[2-4] The finding suggests that the final step of secretion, i.e., exocytotic fusion and fission step, may be the primary rate-limiting step under regulation.

The exocytotic fusion step is energetically unfavorable since the juxtaposed granule and plasma membrane must come into close molecular contact against the short-range of hydration repulsive force.[5] However, the requirement of ATP for secretion in variety of secretory cells was found to be variable from absolute to unnecessary.[6,7] Renin secretion has also been found to be metabolically linked in some studies[8-10] but not in others.[11-14] Such variable requirement of ATP for secretion in general may reflect an indirect coupling of exocytosis to ATP utilization. Thus, ATP may generate and maintain electrochemical or osmotic gradients which provide potential energy for exocytotic process. Indirect coupling of exocytosis via the chemiosmotic gradient has been proposed for regulation of protozoan secretion. The mucocyst in tetrahyma and the trichocyst in paramecium undergo dramatic swelling just prior to or during exocytosis.[15,16] A chemiosmotic involvement in the secretory process is suggested by the observation that hypertonicity of incubation media inhibits secretion in vitro in a variety of secretory cells including JG cells, while hypotonicity stimulates secretion.[2,6,11,17-24]

Osmotic swelling of the secretory granule may be produced by increasing osmotic activity of the granule lumen. This may be achieved either by activation of osmotically inactive macromolecular granule content, or an increased granule membrane permeability or transport of ions. Among these possibilities an inwardly directed ATP-dependent H^+-translocating ATPase(H^+-APTase or H^+-pump) has received much attention because of its occurrence in all secretory granule membranes so far studied.[26] This H^+-ATPase transports H^+ into the secretory granule generating an electrochemical potential such that the inside of the granule is acidic and electrically positive relative to the cytoplasm.[22-26] The coupling of an H^+- and/or voltage-gradient to granule swelling and exocytotic fusion has been termed the chemiosmotic mechanism for secretion.[6] According to this mechanism, the H^+-gradient might be coupled to osmotic swelling of the secretory granule by accumulation of

the monovalent cations(e.g., K^+) via an electroneutral K^+/H^+ exchange[27] or by the accumulation of Cl^- driven by the positive electrical potential.[6]

The present studies were initiated to define whether the nature of the coupling of ATP usage to secretion of renin is direct, or indirect via the generation of an electrochemical gradient. The data obtained strongly support an indirect coupling mediated by an H^+-gradient dependent chemiosmotic force. A preliminary report of some of these results has been presented.[28]

Ⅱ. Methods

Studies on renin secretion in vitro were conducted with renal cortical slices of rabbits fed a low Na^+ diet (Bio-Serve, Inc., Frenchtown, New Jersey, USA)for at least 1 week before the experiments. Renal cortical slices about 0.5mm thick were prepared with a Stadie-Riggs tissue slicer as described previously.[3] They were preincubated in about 100ml of the standard incubation medium continuously gassed with 100% O_2 at 37℃ for 45 to 60min with two or three washings with prewarmed solution. Following the preincubation, renal cortical slices(100 to 200mg wet weight) were incubated in 5ml of the standard, or modified incubation medium. The standard incubation medium(without normal Na^+) contained(in mM) NaCl, 145 ; KCl, 5.0 ; $CaCl_2$, 2.5 ; $MgCl_2$, 1.0 ; glucose, 10 ; N−2−hydroxyethyliperazine-N-2-ethanesulfonic acid(HEPES), 10 ; pH 7.0. When the pH of incubation media was 5.0, 6.0 or 8.0, 10mM Tris-acetate, 2-(N-morpholino) ethanesulfonic acid(MES), or Tris was used, respectively. Hypo-osmotic and isosmotic modified medium was prepared from the standard incubation medium by decreasing the concentration of NaCl to 75mM with(isosmotic) and without(hypo-osmotic) supplementation of 150mM sucrose. High K^+(145mM) medium was prepared from the standard modified incubation medium by substitution of KCl for NaCl. Ca^{2+}-free media were prepared by omission of $CaCl_2$ and inclusion of 1-5mM ethyleneglycol bis(β-aminoethyl ether) N, N, N, N-tetraacetic acid(EGTA).

For experiments with 2-deoxy-D-glucose(2-DG ; 5mM), 6-aminonicotinamide(6-AN ; 0.1 to 1.0mM), KCN(5mM), carbonylcyanide−m−chlorophenylhydrazone(CCCP ; 10^{-5}M) or bafilomycin A_1(10^{-7}-10^{-5} M), a specific inhibitor of vacuole H^+-ATPase inhibitor,[29] slices were incubated for an hour in standard medium, Ca^{2+}-free modified medium, or Ca^{2+}-free modified medium containing a calmodulin antagonist, calmidazolium($5x10^{-5}$M) and then transferred to media of the same composition containing a metabolic inhibitor. For experiments with nigericin, slices were incubated for one hour in Ca^{2+}-free modified incubation medium(with normal Na^+) or high K^+ (145mM) medium, and then transferred to fresh medium of the same composition containing nigericin. For studies with weak bases such as NH_4Cl, benzylamine, chloroquine and methylamine, slices were incubated in Ca^{2+}-free medium without bases in the first hour and then with bases during the second and third hour.

In one series of experiments, slices were incubated without benzylamine during the third hour to see whether effects of benzylamine on renin secretion is reversible. In the experiments with 10 and 30mM bases, the Na^+ concentration of the medium was kept constant at 115mM and isotonicity was maintained by adding sucrose as required. In experiments where osmolality of the incubation medium was altered, slices were incubated for the first hour in Ca^{2+}-free isosmotic modified medium(75mM NaCl+150mM Sucrose),for the second hour in hypo-osmotic(150mOsm /kgH_2O) Ca^{2+}-free medium, and for the third hour in either hypo-osmotic or isosmotic media. In one series of experiments, osmolality of the incubation medium was altered in the presence of CCCP(10^{-5}M). The first hour incubation without testing agents served as the control.

Incubation medium taken at the end of each period

was immediately centrifuged at 2,000xg for 10min at 4℃. The clear supernatant was frozen for the later determination of renin activity. An aliquot of the supernatant was incubated at 37℃ with plasma of nephrectomized rabbits. Angiotensin I(AI) generated was measured with the use of the New England Nuclear Angiotensin I radioimmunoassay kit as described previously.[30] The renal cortical slices at the end of each experiment were blotted on a Whatman filter paper and weighed. The rate of renin secretion is expressed as nanogram of AI per 100mg wet tissue weight per hour(ng AI/100mg/hr) or as the ratio of renin secretion during the experimental period to that of the control period. The significance of the difference between values was evaluated by the Student's t-test.

CCCP, calmidazolium and nigericin were obtained from Calbiochem(San Diego, California, USA), and 2-DG and 6-AN from Sigma Chemicals(St. Louis, Missouri, USA). Bafilomycin A_1 was the kind gift of Stefan Kremer(Universität Tübingen, Germany).

Ⅲ. Result

Effects of metabolic inhibitors on renin secretion.

The effects of various metabolic inhibitors on renin secretion in the standard incubation medium in the presence and absence of Ca^{2+}(2.5mM) are summarized in Table 1. To determine whether inhibition of glycolysis altered renin secretion, slices were incubated with 2-DG in a glucose-free medium supplemented with pyruvate(5mM) to maintain mitochondrial oxidation. 2-DG(5mM) had no effect on renin secretion(Table 1). An inhibitor of hexose monophosphate shunt, 6-AN, not only failed to inhibit but significantly stimulated renin secretion($P<0.005$). On the other hand, KCN and CCCP, inhibitors of the mitochondrial oxidative phosphorylation, significantly inhibited renin secretion between 60-70%($P<0.005$). Bafilomycin A_1($10^{-7}-10^{-5}$M), a potent and specific inhibitor of the electrogenic H^+-ATPase in a variety of secretory granule membrane,[29] had no effect on renin secretion.

The inhibitory effects of KCN and CCCP may have resulted from a number of cellular changes. For example, inhibition of ATP synthesis may have led to an increase in the intracellular Ca^{2+} concentration, and an elevation of the intracellular Ca^{2+} is known to suppress renin secretion.[1, 30, 31] This possibility was tested by determining the effects of KCN and CCCP under condition where the inhibitory effects of Ca^{2+} on renin secretion were excluded by incubating slices in Ca^{2+}-free medium and Ca^{2+}-free medium containing the calmodulin antagonist calmidazolium. As noted in previous studies, [30, 31] slices incubated in Ca^{2+}-free modified Krebs-Ringer bicarbonate(KRB) medium or in the presence of calmidazolium had higher rates of renin secretion(compare control values in Table 1). When slices were incubated in the absence

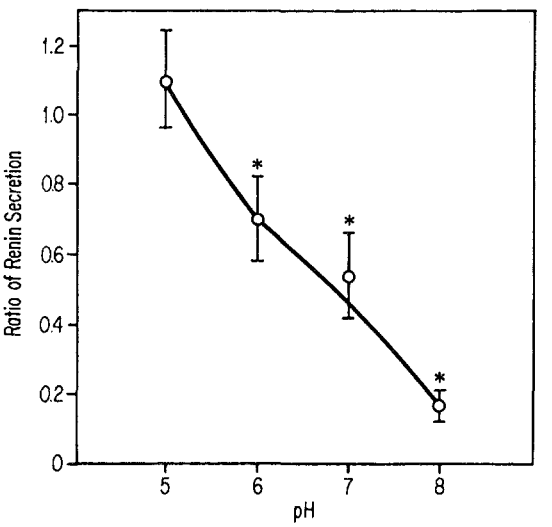

Fig 1. pH-dependent inhibition of renin secretion by CCCP. Slices were incubated in Ca^{2+}-free media at meduim pH 5.0, 6.0, 7.0 or 8.0 during the first hour and then CCCP(10^{-5}M) was added to the media at the start of the second hour. The result is expressed as the ratio of renin secretion during the second hour to that during the first hour. Each point is mean ± S.E. from 5 observations. CCCP inhibits renin secretion by 31±12% at pH 6.0, 46±11% at pH 7.0 and 83±4% at pH 8.0, respectively. Asterisks denote a significant difference from a ratio of 1.0($P<0.05$).

of Ca^{2+}(B, Table 1) or in the presence of calmidazolium(C, Table 1), KCN failed to inhibit renin secretion. On the other hand, CCCP inhibited renin secretion to the same extent(about 70%) regardless of the presence of Ca^{2+} and calmidazolium, suggesting that CCCP may inhibit renin secretion by a mechanism other than limiting ATP synthesis. The rest of experiments were conducted in the Ca^{2+}-free media to exclude potential effects of Ca^{2+} altered by experimental perturbations.

CCCP is a protonphore known to dissipate H^+ gradients across the membranes.[32] To examine whether the inhibitory effects of CCCP on renin secretion is indeed due to dissipation of transmembrane H^+ gradients, we compared its effects at varying H^+ gradients. Cellular H^+ gradients were altered by incubating slices at different medium pH. As shown in Fig. 1, CCCP(10^{-5}M) had no inhibitory effects on renin secretion at medium pH 5.0.

Table 1 Effect of various metabolic inhibitors on renin secretion.

Metabolic inhibitor	Renin Secretion(ng AI/100mg/hr) −inhibitor (I)	+ inhibitor (II)	II/I
A. Ca^{2+}−containing medium			
2−DG(5mM)	59.4± 8.5	47.9± 6.9	0.99±0.16
6−AN(0.25mM)	41.0± 4.7	63.1± 8.3	1.61±0.22*
(1.0mM)	121±10.9	214±16.3	1.80±0.12*
KCN(5mM)	60.7± 9.3	16.1± 6.0	0.33±0.12*
CCCP(10^{-5}M)	62.4±10.1	14.6± 4.8	0.32±0.11*
BafilomycinA_1(10^{-5}M)	74.0±13.8	68.4± 9.2	0.97±0.09
B. Ca^{2+}−free medium			
KCN(5mM)	571±96.8	470±58.3	1.17±0.26
CCCP(10^{-5}M)	755±154	180±26.2	0.32±0.06*
C. Ca^{2+}−free medium +calmidazolium			
KCN(5mM)	1366±179	1252±177	1.05±0.14
CCCP(10^{-5}M)	1582±340	538±119	0.42±0.09*

Values are mean ± S.E from 5-6 incubation flasks. Renal cortical slices were incubated in the standard incubation medium(A), Ca^{2+}-free medium(B) Ca^{2+}-free medium containing 5×10^{-5}M calmidazolium(C) for the first hour [−inhibitor(I)], and in the pressence of metabolic inhibitor for the second hour [+Inhibitor(II)]. II/I is the ratio of renin secretion rates during the two incubation periods. These ratio values were corrected for the spontaneous changes in renin secretion rate observed in control slices. *Significantly different from unity($P<0.05$ or smaller).

Effects of nigericin on renin secretion.

The results of the preceeding series of experiments with CCCP suggest that the transmembrane H^+-gradient may play an important role in renin secretion. To explore this possibility further, the effects of nigericin on renin secretion were determined. Nigericin is a carboxylic ionophore which promotes an electroneutral exchange of K^+ for H^+ along their concentration gradients.[33] Nigericin at 1.0×10^{-5}M had no effect(data not shown) but at 5×10^{-5}M significantly stimulated renin secretion on Ca^{2+}-free standard medium($Na^+=145$mM)($P<0.01$, Table 2).

Table 2. Stimulation of renin secretion by nigericin in high Na^+ and K^+ medium.

Medium	Renin Secretion(ng AI/100 mg/hr) Control	+ Nigericin	Ratio
High Na^+	52.9±13.7	212±86.7	3.70±0.65
High K^+	399±107	1351±418	4.33±0.66

Values are mean ± S.E. from 6 incubation flasks for each group. Renal cortical slices were in the absence(Control) and then presence of nigericin(5×10^{-5}M) in a Ca^{2+}-free high Na^+(145mM) or high K^+(145mM) incubation medium at pH 7.0 for one hour each. Ratio is the renin secretion rate of nigericin/control. The increase in renin secretion by nigericin was significant($P<0.01$).

The next series of experiments was repeated in Ca^{2+}-free, high K^+(145mM) medium at pH 7.0 to minimize or abolish both K^+-and H^+-gradients across the plasma membrane. The stimulatory effect of nigericin on renin secretion was still apparent which was comparable in magnitude to that in Na^+ medium(Table 2). These results support the possibility that the H^+-gradient across intracellular organelle membranes may indeed play a role in renin secretion.

Effects of weak bases on renin secretion.

Weak bases such as NH_4Cl are known to accumulate in acidic intracellular compartments in a H^+-gradient dependent manner and to cause osmotic swelling as does nigericin. As summarized in Table 3, ammonium chloride, methylamine, benzylamine and chloroquine all produced concentration-and time-dependent stimulation of renin secretion. Among the four weak bases tested, chloroquine was the most potent in stimulating renin secretion : the magnitude of stimulation by 0.2mM chloroquine was comparable to that by 30mM of the other three weak bases. This result is in concert with observations that chloroquine has greater effects on osmotic swelling of intracellular acidic organelles than other weak bases.[34, 35]

Table 3. Effects of weak bases on renin secretion.

Weak Base	Conc. (mM)	N	Renin Secretion(Ratio to the Control Period) Period Ⅱ	Period Ⅲ	Period Ⅲ/Ⅱ
Ammonium Chloride	10	6	1.12±0.03	1.41±0.18	1.25±0.15
	30	11	1.21±0.05	1.98±0.18	1.68±0.17
Methylamine	10	6	1.16±0.08	1.43±0.07	1.26±0.08
	30	12	1.46±0.13	2.14±0.25	1.48±0.13
Benzylamine	10	6	1.24±0.05	1.37±0.03	1.11±0.03
	30	12	1.78±0.14	2.90±0.21	1.76±0.20
Chloroguine	0.2	6	1.20±0.08	2.38±0.18	1.99±0.14

Values are mean±S.E. Renal cortical slices were incubated in the Ca^{2+}-free modified incubation medium during the first hour without a weak base(Control Period Ⅰ) followed by the second(period Ⅱ)and third hour(Period Ⅲ)with a weak base. Renin secretion during period Ⅱ and Ⅲ was significantly greater than that of control period Ⅰ ($P<0.05$ or smaller). Ⅲ/Ⅱ values are also significantly greater than 1.0 except that with 10mM ammonium chloride.

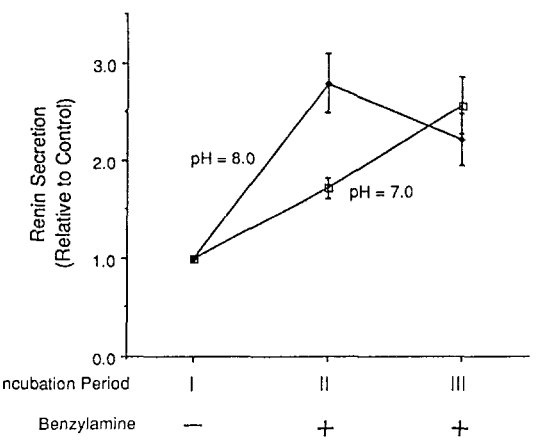

Fig 2. pH-dependent stimulation of renin secretion by benzylamine. Slices were incubated in an isosmotic Ca^{2+}-free medium at pH 7.0 or 8.0 for three hours. Benzylamin(30mM) was included during the second and third hour. NaCl concentration of the incubation media was kept constant at 115mM and isotonicity was maintained by including 60mM sucrose as required. Results are expressed as the ratio of renin secretion during the second and third hour in the pressence of benzylamine to that during the first hour without the weak base. Each point is the mean± S.E. from 6 observations. The ratio values at both pH were significantly greater than unity($P<0.005$).

Weak bases cross biological membranes by diffusion of unprotonated uncharged forms and the concentration of which is determined by pH.[34, 35] In one series of experiments, incubation was made with 30mM benzylamine at medium pH 7.0 and 8.0 to vary the concentration of unpronated benzylamine 10-fold.

As seen in Fig. 2, the weak base stimulated renin secretion by 71±11(N=6, $P<0.001$) and 156±29% ($P<0.001$) at pH 7.0 and 179±30%(N=6. $P<0.001$) and 121±27%($P<0.005$) at pH 8.0, one and two hour exposure to the weak base, respectively.

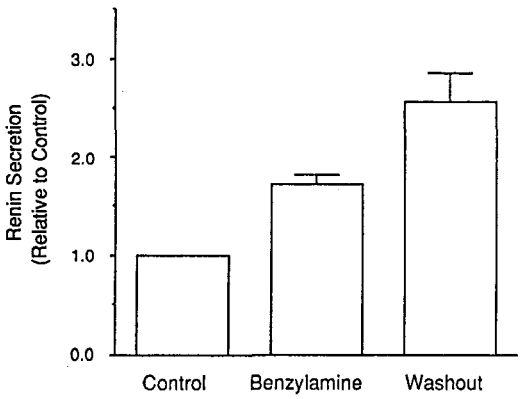

Fig 3. Reversiblity of the stimulation of renin secretion by benzylamine. Slices were incubated in isosmotic medium at pH 7.0 during the first hour as the control. Slices were then incubated in the isosmotic medium containing 30mM benzylamine during the second hour and then isosmotic medium without benzylamine during the third hour. Renin secretion during the second and third hour to that during the first control period was 1.78±0.14 ($P<0.001$, $N=6$) and 2.56±0.29 ($P<0.001$), respectively.

Thus, the stimulation at pH 8.0 was rapid, reaching an apparently maximal level within the first hour of exposure. This increase was significantly greater than that at pH 7.0 during the first hour ($P>0.001$) but not during the second hour of exposure ($P>0.05$). Incubation of slices with 30mM benzylamine during the second hour significantly stimulated renin secretion (Fig. 3, $P<0.001$, $N=6$).

When slices were incubated in the absence of benzylamine during the third hour, renin secretion stimulated by benzylamine during the second hour was not reversed to the control level, suggesting that the stimulation is not readily reversible.

Effects of hypo-osmolality on renin secretion.

Incubation of cells with nigericin and weak bases would produce osmotic swelling of intracellular acidic organelles. Since renin granules may have acidic interior,[37] it follows that swelling of renin granule might be responsible for increased renin secretion produced by these agents. To test this possibility, we produced swelling of intracellular organelles by incubation of slices in hypo-osmotic media. Incubation in a Ca^{2+}-free hypo-osmotic medium stimulated renin secretion by 314±35% ($P<0.001$, $N=5$, left panel of Fig. 4). The stimulated renin secretion was sustained for at least 2 hours. The stimulatory effect of hypotonicity was reversed upon returning the slices to an isosmotic medium ($P>0.2$, $N=6$, right panel of Fig. 4)

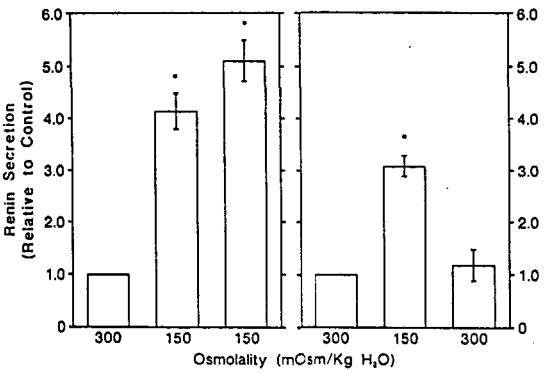

Fig 4. Reversibility of the stimulation of renin secretion by incubation in hypo-osmotic medium. Slices were incubated for the first hour in an isosmotic Ca^{2+}-free modified KRB containing 75mM NaCl made isosmotic by addition of 150mM sucrose. One group of slices was then incubated for two consecutive 1 hr periods in a hypo-osmotic medium containing 75mM NaCl and no sucrose (left panel). Another group of slices was incubated in the hypo-osmotic medium during the second period and the isosmotic medium during the third period (right panel). Results are expressed as the ratio of renin secretion during the second and third periods to that during the first control period. Results are the mean± S.E. from 5 (left panel) and 6 (right panel) observations. Asterisks denote significant difference from a ratio of 1.0 ($P<0.05$).

To examine whether CCCP affects renin secretion when secretion is induced in an H^+-gradient independent manner, the stimulatory effects of hypo-osmolality were studied in the absence and presence of CCCP (10^{-5}M) in a parallel fashion. Incubation of tissues in hypo-osmotic media significantly increased

secretion in the absence of CCCP(left panel of Fig. 5 ; 809±81%, P<0.001, N=6). In the presence of CCCP incubation in a hypo-osmotic medium stimulated renin secretion to nearly the same extent(right panel of Fig. 5 ; 647±120%, P<0.001, N=6). The difference in the relative increases of renin secretion is not significant(P>0.05 by unpaired Student's t-test).

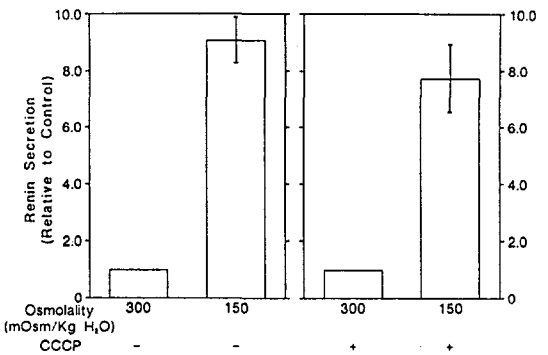

Fig 5. Effect of CCCP on the stimulation of renin secretion by hypo-osmolality. The experimental procedures were the same as described in the legend for Fig. 3(left panel) except that one group of tissues was incubated in the presence of CCCP (10^{-5}M). In the absence of CCCP, lowering medium osmolality significantly stimulated renin secretion by 809±81% (126.1±31.4 to 1,093±218ng AI/100mg/hr;P<0.001, N=6;left panel). In the presence of CCCP, lowering medium osmolality stimulated renin secretion by 674±120% (from 78.8±18.5 to 541±81ng AI/100mg/hr; P<0.001, N=6; right panel) in the presence of CCCP. The difference in the relative increases of secretion is not significant(P<0.05 by unpaired t-test).

Ⅳ. Discussion

The present studies provide several lines of evidence as to the nature of the indirect coupling of ATP to renin secretion. Inhibition of ATP synthesis by inhibiting glycolysis with 2-DG did not affect renin secretion(Table 1). Other investigators also noted that renin secretion was unaffected when glycolysis was inhibited by sodium iodoacetate or sodium fluoride.[8, 11, 13] On the other hand, it has been reported that renin secretion rate and renal renin content are diminished by removal of glucose from the medium or by the addition of an inhibitor of the hexose monophosphate shunt, 6-AN.[10] We observed that 6-AN stimulated renin secretion(Table 1). Our finding is in agreement with studies on parathyroid hormone secretion in that the rate of secretion is reciprocal to flux through the shunt pathway.[36] The reason(s) for the conflicting results from studies with renal cortical slices and isolated kidneys is unknown.

Inhibition of mitochondrial ATP synthesis with KCN inhibited renin secretion(Table 1). However, this inhibition of secretion by KCN was no longer observed when slices were incubated in a Ca^{2+}-free medium(Table 1) or in a Ca^{2+}-free media in the presence of a calmodulin inhibitor, calmidazolium(Table 1). Lyons also found in rat renal cortical slices that KCN inhibits renin secretion only in the presence of Ca^{2+} *in incubation medium.*[13] *Since an elevated intracellular* Ca^{2+} concentration inhibits renin secretion,[1, 14, 31] it is most likely that the inhibitory action of KCN on renin secretion is secondary to an increased intracellular Ca^{2+} concentration. Other studies have, however, failed to show inhibition of renin secretion by cyanide even in the presence of the extracellular Ca^{2+} [8, 11] The reason for the failure of cyanide to inhibit renin secretion in those earlier studies is unclear. Nevertheless, taken together, neither the results of those earlier studies nor those of the present studies support the notion that ATP derived from glycolysis or oxidative phosphorylation is obligatory for the renin secretory process as found in other secretory systems.[6, 7]. The recent finding of ATP-independent secretion from a variety of secretory cells led to the view that ATP has an modulatory role in maintaining the exocytosis in "primed" state.[6]

Unlike KCN, CCCP inhibited renin secretion even when the inhibitory effect of Ca^{2+} on renin secretion was excluded(Table 1). CCCP was reported to inhibit secretion also in a variety of secretory cells where Ca^{2}

$^{+}$ is the key stimulatory secretion signal.[16, 17, 25] Thus, the inhibition of secretion by CCCP cannot be accounted for by elevation of Ca^{2+}, suggesting that this protonophore may exert additional inhibitory effects on the renin secretory process. It is generally accepted that proton ionophores such as CCCP and carbonylcyanide - p(Tri - fluoromethoxy) - phenylhydrazone(FCCP) exert their effects by increasing the transmembrane flux of H^+, thereby tending to collapse the transmembrane H^+ electrochemical gradients.[32] The possibility that this action of CCCP underlies its inhibitory effect on renin secretion is supported, albeit indirectly, by the finding that the inhibitory effect of CCCP on renin was H^+-gradient dependent (Fig. 1). It is noteworthy that CCCP had no effect on renin secretion which was stimulated in a H^+-gradient independent manner by hypo-osmolality(Fig. 5). This result as well as the H^+-gradient dependent action of CCCP makes it unlikely that CCCP inhibits renin secretion by non-specific toxic effects.

CCCP did not inhibit renin secretion at medium pH 5.0 but did inhibit at pH 7.0 which is very close to the estimated cytosolic pH(7.06)[36] and there was no appreciable H^+-gradient across the plasma membrane. Furthermore, the stimulatory effect of nigericin was still apparent in a high K^+ (145mM) medium at pH 7.0(Table 2) where the gradients for H^+ and K^+ across the plasma membrane were abolished. Therefore, the effects of CCCP and nigercin are likely the result of their action on H^+ and K^+-gradients across intracellular organelles rather than the plasma membrane.

Many intracellular organelles, including secretory granules, maintain a steep H^+ gradient by an ATP dependent electrogenic H^+-ATPase.[6, 22-26] It has been reported that weak bases included in chloroquine can accumulate within the renin granules, suggesting that the interior of the renin granule is acidic.[37] If the renin granule indeed contains an electrogenic H^+-ATPase, by analogy with other types of secretory granules, CCCP may inhibit renin secretion by dissipating the H^+ gradient across the renin granule as it does in other secretory granules.[6, 17, 25] On the contrary, inhibition of the H^+-ATPase by bafilomycin A_1 did not inhibit renin secretion(Table 1). This result suggests that the renin secretory granule membrane has a low permeability to H^+ and the established H^+-gradient may dissipate very slowly with little effect on renin secretion.

A chemiosmotic mechanism driven by the H^+- and K^+-electrochemical gradients across the renin secretory granule membrane could explain not only the inhibitory effect of CCCP but also the stimulatory effects of nigericin and weak bases on renin secretion. Given the high concentrations of K^+ in the cytosol and H^+ in the granule interior, nigericin would promote an electroneutral exchange of H^+/K^+, thereby effecting an intragranular accumulation of osmotically active K^+ with subsequent granule swelling.[34, 35] Week bases equilibrate across biological membranes by diffusion of unprotonated neutral forms. In acidic intracellular organelles, such bases become protonated and accumulate since the protonated forms are less permeant to the membrane.[34, 35] Thus, an accumulation of weak bases with attendant anions to high concentration could cause osmotic swelling of acidic granules.[34, 35]

The possibility of a chemiosmotic mechanism in renin secretion is further supported by the finding of reversible stimulation of renin secretion by incubation in hypo-osmotic medium(Fig. 5). Our findings of stimulation of renin secretion in renal cortical slices by nigericin, weak bases and hypo-osmotic incubation medium are consistent with those in isolated glomeruli[2, 11, 12, 20] and afferent arterioles[21]. It is important to note that our experiments were conducted in rabbit renal cortical slices incubated in Ca^{2+}-free medium and the stimulation of renin secretion by nigericin, weak bases and hypo-osmolality was sustained. Experiments with isolated rat glomeruli[2, 11, 12, 20, 38, 39] were done in media containing Ca^{2+}, and the stimulation was transient, sometimes with delayed in-

hibition.[35, 39] Such differences could be attributable to species difference(rat vs rabbit), but in part to the Ca^{2+} in the incubation medium(see below).

Several lines of evidence from seemingly diverse experiments support the hypothesis that chemiosmotic swelling of the renin secretory granule may increase the rate of renin secretion in vitro. However, whether such a chemiosmotic mechanism and granular swelling is involved in the physiological regulation of renin secretion cannot yet be addressed. Physiological secretagogues such as cholecystokinin and secretin activate K^+ and Cl^-transport across the pancreatic zymogen granule membrane, resulting in net influx of KCl and water into the granules, thereby causing osmotic swelling.[40] As noted above, in the presence of Ca^{2+} in incubation medium, stimulation of renin secretion by hypotonicity was completely blocked(unpublished observation) or less in magnitude than in its absence.[20] Since Ca^{2+} is the well established intracellular inhibitory singnal for renin secretion,[1, 14, 28, 31] if granular swelling is involved in the physiologic control of renin secretion, Ca^{2+} may act by inhibiting osmotic swelling of the granule. A word of caution is necessary, however. Studies on the catecholamine secretion from chromaffin cells have presented evidence that the electrochemical gradient of H^+ across the chromaffin granule membrane is not essential for catecholamine secretion.[41, 42] It is also controversial whether chemiosmotic swelling of secretory granules is obligatory for fusion or subsequent exocytotic steps.[43]

In summary, the present studies indicate that renin secretion may be regulated by ATP indirectly by the generation of a H^+ gradient across the renin granule which could be equivalent to the "ATP-induced primed state" of exocytosis.[7] This gradient may then be used as a driving force for the accumulation of ions and consequent swelling of the granules and this swelling may promote exocytosis in some manner. More direct evidence of the presence of the postulated H^+-ATPase and other mechanisms in the renin secretory granule will be required to support or deny this hypothesis as well as the relevance of this mechanism to the physiological control of renin secretion.

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＝국문초록＝

Renin 분비의 화학삼투압적 조절

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본 실험에서는 토끼의 신피질 Slices를 이용하여 renin분비와 세포 대사과정 사이의 연관관계를 살펴보았다. 2-deoxy-D-glucose로 해당과정을 억제한 경우에는 renin분비에 변화가 없는 반면, 6-aminonicotinamide를 사용하여 hexose monophosphate shunt를 억제했을 때는 renin분비가 촉진되었다. 그러나, 미토콘드리아에서 APT생성을 억제한다고 알려진 KCN과 carbonylcyanide-m-chlorophenylhydrazone(이하 CCCP)는 renin분비를 억제하였다. 세포배양액에서 Ca^{2+}을 제거할 경우 KCN에 의한 renin 분비억제 효과가 상실되는 것으로 보아 KCN은 세포내 Ca^{2+}을 증가시키고 이에 따른 2차적인 현상으로 renin 분비가 억제되는 것으로 생각된다. 반면, CCCP에 의한 억제효과는 세포외액에서 Ca^{2+}을 제거하거나 혹은 calmodulin antagonist인 calmidazolium을 첨가하여도 영향을 받지 않으므로, CCCP는 막내외의 H^+－농도차를 없애므로써 renin분비를 억제하는 것으로 보인다. 그러나 H^+-ATPase억제제인 bafilomycin A1은 효과가 없었다. 따라서 막내외의 H^+ 농도 차이와 renin분비 사이의 관계를 H^+/K^+ exchange ionophore인 nigericin을 사용하여 살펴보았다. Renin분비가 nigericin에 의해 크게 촉진되는 것으로 보아 H^+이나 다른 일가양이온의 농도차가 renin분비에 중요한 역할을 함을 알 수 있었다. 막 내외 H^+ 농도차에 의해 세포내 산성 vesicle내에 축적될 것으로 생각되는 ammonium chloride, benzylamine, chloroquine, methylamine등의 약염기도 renin분비를 촉진하였다. 신피질 slices를 hypo－osmotic medium에 배양시킨 경우 renin분비가 가역적으로 촉진되었다. 이상의 결과는 ATP는 renin granular membrane에 있어서의 H^+ 혹은 다른 이온의 농도차를 유발 또는 유지함으로써 renin 분비에 간접적으로 영향을 미칠 가능성을 시사한다. 또한, 이러한 이온의 농도차는 osmotic swelling에 의한 분비와 연관되어 있을 것으로 생각된다.

Key Words : ATP, Proton－gradient, Osmotic swelling, Renin secretory granules, Stimulus－secretion coupling