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EFFECT OF ATRIAL NATRIURETIC PEPTIDE ON RENIN RELEASE IN RAT ISOLATED GLOMERULI

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SUMMARY: Effects of atrial natriuretic peptide (ANP) on renin release in isolated rat glomeruli were investigated. ANP suppressed renin release by 25 % at $5x10^{-8}$ M when glomeruli were incubated in a medium containing 1.26 mM calcium (p=0.0019). When glomeruli were incubated in a calcium free medium containing 2 mM EGTA, ANP suppressed stimulated renin release significantly at $5x10^{-8}$ and $5x10^{-9}$ M by 25 % (p=0.0204, and p=0.0101, respectively). These results indicate that ANP suppresses renin release in a dose dependent manner, probably through a calcium independent process.
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Atrial natriuretic peptide (ANP) is secreted from atrial myocytes and has various effects on circulation, i.e. natriuresis, diuresis, suppression of renin-aldosterone system, etc. ANP suppresses plasma renin activity consistently in vivo (1). The simplest explanation for this is an increased sodium load to macula densa. It has also been known that ANP suppresses plasma renin activity in animals with a non-filtering kidney, suggesting a direct effect of ANP on renin-secreting cells without mediating the macula densa mechanism (2). So far, reports on the direct effects of ANP on renin release in in vitro studies are quite conflicting. ANP suppressed basal renin release (3, 4), increased renin release (5, 6), and had no effect (7, 8, 9, 10). Intracellular mechanisms of ANP on renin release have not yet clearly elucidated. ANP suppressed increased renin release caused by decreased intracellular calcium concentration (3), and

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ABBREVIATIONS: ANP, atrial natriuretic peptide; AP III, atriopeptin III.

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had no effect on it (9). On the other hand, increased renin release by cAMP mechanism was suppressed (3, 9, 11), and not affected (6, 7, 10) by ANP. ANP enhanced the suppressive effect of angiotensin II on renin release (7), and showed no effect (6). ANP stimulated renin release in a cGMP independent process (5), and suppressed renin release by cGMP dependent process (4).

It has been shown that ANP inhibits calcium release from sarcoplasmic reticulum in vascular smooth muscle cells (12), and calcium is an inhibitory intracellular second messenger in renin release (13). Therefore, we focused on calcium mechanism in order to clarify the so far conflicting effects of ANP upon renin release using isolated rat glomeruli.

MATERIALS AND METHODS

MATERIALS

The following materials were obtained as follows; ANP from Peninsula Laboratories (Belmont, CA, USA), bovine serum albumin (BSA) from Sigma Chemicals Co. (London, U.K.), and angiotensin I radioimmunoassay kit from CIS (Saluggia, Italy). Sheep renin substrate was a gift from Tsukuba University (Ibaraki, Japan).

ISOLATION OF GLOMERULI

A male Wistar rat weighing 200 to 300 g was decapitated under light ether anesthesia. Glomeruli were isolated by sieving and centrifugation by a modified method of Misra (14). Briefly mentioned, kidneys were taken immediately after decapitation and immersed in ice cold Hank's solution (NaCl 137 mM, KCl 5.4 mM, $MgSO_4.7H_2O$ 0.4 mM, Na_2HPO_4(anhy) 0.34 mM, $CaCl_2.2H_2O$ 1.26 mM, $NaHCO_3$ 4.17 mM, KH_2PO_4 0.44 mM, $MgCl_2.6H_2O$ 0.49 mM, glucose 5.56 mM) containing 0.2 % BSA with pH being adjusted to 7.40. Then kidneys were dissected and medulla were removed. Cortical tissues were minced to a paste-like consistency. These materials were sieved through a 250-micron stainless mesh and sieved materials were collected into ice cold oxygenated Hank's solution. The suspension was sieved through 150-micron nylon mesh excluding large tissue debri. The suspension was centrifuged at 10 G 3 times each for 3 minutes. The suspension was sieved through 125 micron nylon mesh. The suspension was centrifuged again. Glomeruli collected on 75 micron nylon mesh were suspended in ice cold oxygenated Hank's solution. The final pellet consisted of almost pure glomeruli without tubular contamination.

SUPERFUSION OF ISOLATED GLOMERULI

700 isolated glomeruli were put in a chamber and superfused with a syringe pump (Nikkiso, Tokyo, Japan) at a rate of 1 ml/h in a 37℃ incubator. Glomeruli were preincubated for 45 minutes, and thereafter superfusate was collected at 15-minute intervals for 90 minutes. A syringe was changed from one containing Hank's solution to relevant syringes. The renin activity during the first 15-minute interval served as a baseline and was normalized as 100 %. Samples between 15 minutes and 30 minutes were not analyzed, because data were not reliable due to mechanical stimulation by a change of a syringe. Renin activities from 30 to 90 minutes were expressed as percentage of the baseline.

(1)Effect of ANP on basal renin release

After 15-minute baseline period incubation medium was changed to Hank's solution containing $5x10^{-10}$, $5x10^{-9}$, or $5x10^{-8}$ M ANP. 2 chambers were incubated simultaneously, one of which served as a time control. In order to avoid an influence due to a change of incubation medium, a syringe was also changed in a time control. 6 experiments were done for each ANP concentration.

(2)Effect of ANP on stimulated renin release due to calcium chelation

Same as the above experiment, after 15-min baseline period incubation medium was changed to calcium free solution containing 2 mM EGTA, otherwise electrolytes and glucose content were the same as Hank's solution. ANP concentration were $5x10^{-10}$, $5x10^{-9}$, and $5x10^{-8}$ M. 2 chambers were incubated simultaneously, one of which was served as a time control. Calcium free solution containing 2 mM EGTA without ANP was used in a time control. 6 experiments were done for each ANP concentration.

RADIOIMMUNOASSAY OF RENIN ACTIVITY

Renin activities in incubation medium were determined by measuring the generated angiotensin I under the existence of excess renin substrate using radioimmunoassay kit. Renin substrate was obtained by purifying plasma of a sheep in which bilateral nephrectomy had been done 72 hours previously. Renin substrate contains angiotensinogen which is equivalent to 540 ng angiotensin I/mg (15).

Statistical analysis was done by repeated measures of analysis of variance.

RESULTS

(1) Effect of ANP on basal renin release

Renin activity declined along with time from 100 % during baseline period to about 60 % during 45 to 90 minutes in time controls (Fig. 1). ANP did not exert its effect on renin release either at $5x10^{-10}$ or $5x10^{-9}$

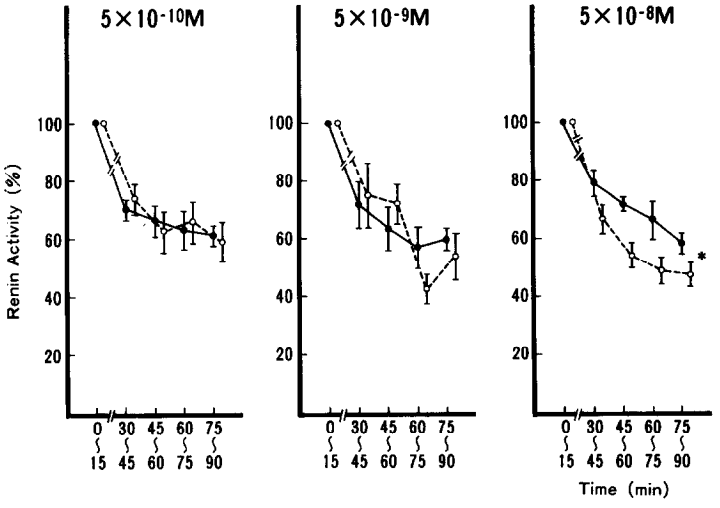

Fig. 1 Effect of ANP on basal renin release. Open circles show ANP and closed circles show time control. Data show mean ± SEM.
*:p=0.0019

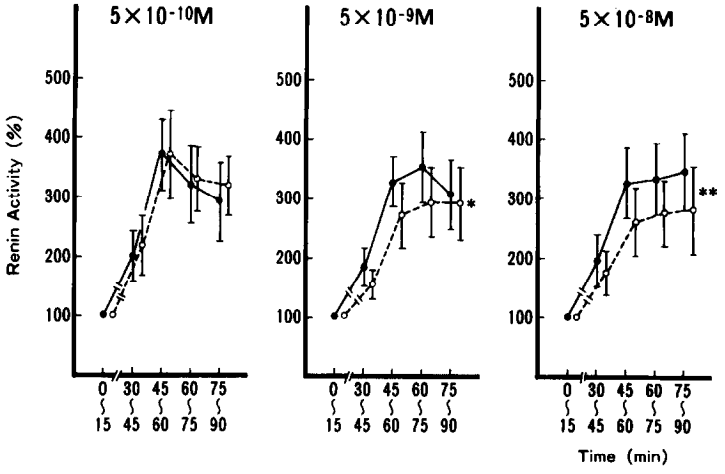

Fig. 2 Effect of ANP on stimulated renin release by Ca^{2+} chelation. Open circles show ANP and closed circles show time control. Data show mean ± SEM. *:p=0.0204, **:p=0.0101

M. However, $5x10^{-8}$ M ANP suppressed renin release significantly by about 25 % (p=0.0019).

(2)Effect of ANP on stimulated renin release

Renin release increased to about 350 % of the baseline during 45 to 90 minutes (Fig. 2). $5x10^{-10}$ M ANP did not have any effect on renin release, while $5x10^{-9}$ and $5x10^{-8}$ M ANP suppressed renin release significantly (p=0.0204 and p=0.0101, respectively).

DISCUSSION

Consistent results have not yet been obtained regarding in vitro effect of ANP on renin release. There are 2 intracellular second messengers on renin release, namely Ca^{2+} and cAMP (13). Since ANP has been shown to inhibit Ca^{2+} release from sarcoplasmic reticulum in vascular smooth muscle cells (12), we focused on calcium mechanism of ANP on renin release. Effect of ANP on stimulated renin release by calcium chelation has not yet been done.

In our experiment ANP suppressed both basal and stimulated renin release through calcium chelation in a dose-dependent manner, suggesting that the inhibitory effects of ANP are calcium independent. These results are compatible with a previous report that ANP inhibits renin release stimulated either by calcium free medium or diltiazem at the concentrations

of 10^{-5} and 10^{-6} M, respectively, in renal cortical slices (3). If ANP inhibits Ca^{2+} release from endoplasmic reticulum also in renin secreting cells, ANP should increase renin release. However, this was not the case. Therefore, processes other than Ca^{2+} seem to be involved. Although it has been shown that ANP inhibits Ca^{2+} release from sarcoplasmic reticulum in vascular smooth muscle cells (12), atriopeptin III (AP III) failed to show a change in intracellular Ca^{2+} concentration in cultured JG cells when measured by quin-2 fluorescence (4). AP III has been reported to enhance the suppression of renin release by angiotensin II, while it has no effect on basal renin release (7). However, others failed to show effects of AP III either on stimulated renin release by diltiazem or TMB-8, or suppressed renin release by angiotensin II (9).

cAMP is another intracellular second messenger in renin release. ANP or AP III suppressed stimulated renin release induced by isoproterenol or forskolin (3, 9, 11), while others showed no effect (6, 7, 10). Here, again, results on renin release through cAMP mechanism are not consistent.

ANP increases intracellular cGMP in various tissues (16). It has been shown that M&B 22948, a cGMP specific phosphodiesterase inhibitor, enhances the suppressive effect of AP III on renin release, and methylene blue, a guanylate cyclase inhibitor, inhibits the suppressive effect of AP III, showing that the suppressive effect of AP III is cGMP dependent process (4). On the other hand, another study shows that ANF increased renin release in renal cortical slices in a cGMP independent manner (5). 8bcGMP failed to show a change in renin release in renal cortical slices or in dispersed JG cells (6).

Roles of cGMP and cAMP in the mediation of the effect of ANP on renin release must await further study.

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