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# INHIBITORS OF ALDOSTERONE SECRETION

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**Summary**—Aldosterone secretion may be inhibited by potassium depletion, inhibitors of the renin–angiotensin system, dopamine and atrial natriuretic factor. The latter appears to be an important physiological regulator of aldosterone secretion. ANF inhibits basal, ACTH, Angiotensin II and potassium-stimulated aldosterone production *in vitro* by a direct action on the adrenal gland. *In vivo* data also support a direct inhibitions of aldosterone. The stimulation of aldosterone secretion by infusions of Angiotensin II and potassium is inhibited by simultaneous infusions of ANF. Infusions of ANF lower the basal aldosterone secretion in man.

The mechanism by which ANF inhibits aldosterone is not known. No unifying first step has been identified to explain ANF's ability to inhibit all stimuli. *In vivo*, part of the lowering of aldosterone levels may be due to inhibition of renin secretion. This effect of ANF upon renin is inconsistent and appears to depend upon the experimental conditions.

## INTRODUCTION

A number of earlier studies suggested that an inhibitory system also regulated aldosterone secretion. Mills *et al.* in 1958 [1] noted increased aldosterone secretion in dogs during constriction of the thoracic inferior vena cava, but when the constriction was released the secretory rate for aldosterone fell even below the basal level. Sectioning the cervical vagi did not affect the increase in secretion but prevented the decrease. In 1959, Anderson *et al.*[2] described experiments in which stretching of the right atria inhibited aldosterone secretion. Further studies [3, 4] also supported an inhibitory system that regulated aldosterone production. This inhibitory system was thought to be due to an unknown central reflex mechanism.

In addition, there are other recognized mechanisms inhibiting aldosterone production. Potassium depletion has been known for years to inhibit aldosterone production in response to a variety of stimuli [5]. Inhibitors and the renin–angiotensin system, such as beta blockers or converting enzyme inhibitors, also lower aldosterone production. More recently, dopamine has been suggested as a tonic inhibitor of aldosterone production, since removal of dopamine action through use of dopamine antagonists leads to an increased secretion of aldosterone [6]. Whether dopamine inhibits aldosterone secretion by a central mechanism or a peripheral action is not clear. Calcium channel blockers, at least *in vitro*, inhibit aldosterone production [7, 8]. The influx of calcium seems to play a role in the response of aldosterone secretion to a variety of stimuli. However, the effect *in vivo* of these calcium channel blockers is inconsistent.

Atrial natriuretic factor appears to be an important physiological inhibitory system of aldosterone regulation, and may explain the earlier results of Mills *et al.*[1] and Anderson *et al.*[2].

## EXPERIMENTAL

### *In vitro studies*

Dispersed glomerulosa and fasciculata cells were used *in vitro* as described previously[9]. Briefly, the adrenal glands were removed, defatted, and capsular cells separated by standard techniques; these cells containing primarily glomerulosa cells were approximately 90–95% pure. The inner zone contains fasciculata medullary cells. Incubation is carried out in medium 199 with or without the addition of various stimulators. Aldosterone is measure in the media as an index of steroid production by the glomerulosa cells and corticosterone as an index of production by the fasciculata cells. Synthetic Atriopeptin I, II, and III were obtained from Peninsula Laboratories (Belmont, CA). Synthetic Angiotensin II and ACTH were obtained from Sigma Chemical Company (St Louis, MO).

### *In vivo studies*

The technique of atriopeptin infusions on aldosterone production in rats was described previously [9]. Briefly, atriopeptin was infused into conscious female Sprague–Dawley rats maintained on a normal sodium diet. Polyethylene catheters were implanted into the right common carotid artery and internal jugular vein 48 h before the experiment. The catheters were led subcutaneously to the back of the neck, exteriorized, and filled with heparinized saline. Two h before the experiment the rats were given dexamethasone phosphate 1 mg/kg intraperitoneally to inhibit ACTH release during the experiment. At zero time a small amount of blood was taken from the carotid catheter and the blood loss was replaced with the same volume of isotonic saline. Subsequently, angiotensin or potassium was infused continuously through the jugular catheter with or without an infusion of atriopeptin. Plasma

aldosterone was measured before and after the infusion. Plasma renin activity (PRA), corticosterone, potassium, and hematocrit were determined at the end of the infusion.

*Human studies*

Synthetic atrial natriuretic factor, Met-ANF 8-33 (Merck Sharp & Dohme Research Laboratories, Rahway, NJ) was infused for 4 h with a dose of 0.7 μg/min into 6 hypertensive patients. This was a double-blind crossover experiment in which a placebo was infused the day before or day after the ANF. Blood pressure was measured every 2 min and PRA and plasma aldosterone were measured at 0, 2, 4, 6, and 24 h after the start of the infusion. Other variables measured were urine and plasma electrolytes, plasma ADH, cortisol, and catecholamines.

*Calcium efflux studies*

Separated rat adrenal glomerulosa cells were first pre-labeled with calcium-45, then placed on a Sephadex-G 15 column and superfused with Krebs' Ringer bicarbonate buffer. Atriopeptin, Angiotensin II or potassium was added to the superfusate. The eluate was collected in a fraction collector at 2-min intervals and the efflux of calcium from the pre-labeled cells as well as aldosterone production were measured in the effluent. The technique is similar to that of Kojima *et al.*[10].

Statistical significance was determined by Student's *t*-test and, where appropriate, one-way analysis of variance, and Scheffe's multiple range analysis. A $P$ value of $<0.05$ was considered significant.

## RESULTS

### In vitro

The effect of Atriopeptin I, II, and III on basal aldosterone production by rat adrenal glomerulosa cells was similar to that seen in our previous report of crude atrial extract [11]. All three peptides inhibited the basal aldosterone production; Atriopeptin II and III were more potent than Atriopeptin I. Figure 1 shows the effect of Atriopeptin II on aldosterone production stimulated by ACTH. The addition of $10^{-10}$ M Atriopeptin II inhibited the basal level of aldosterone and shifted the dose–response curve to the right. However, with the higher dose of ACTH the inhibition is overcome. The effect of Atriopeptin II on aldosterone production stimulated by Angiotensin II is shown Fig. 2. There was a significant increase in aldosterone production in response to Angiotensin II. When Atriopeptin II was added, basal production was diminished and the response to Antiotensin II was markedly diminished. This inhibition, in contrast to that of ACTH, cannot be overcome by higher doses of Angiotensin II. The inhibition of steroidogenesis by atriopeptin was specific for the glomerulosa cell in the rat. Corticosterone production was increased in dose-dependent fashion by ACTH stimulation of fasciculata cells and atriopeptin did not inhibit basal production of corticosterone nor the stimulation by ACTH [9].

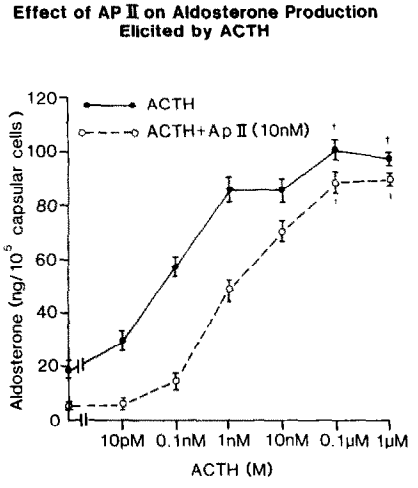


Fig. 1. Aldosterone production by ACTH with or without Atriopeptin II. Each point represents the mean ± SE of 6 experiments done in duplicate, except where indicated by † where $n = 3$. In each experiment, ACTH concentrations are expressed as M/l of incubation medium.

### In vivo

Figure 3 shows the results of experiments in which simultaneous infusion of Atriopeptin II and Angiotensin II upon aldosterone were studied. Angiotensin markedly increased plasma aldosterone levels. When Atriopeptin II was infused simultaneously, there was a marked suppression of the aldosterone response to Angiotensin II. Serum corticosterone, potassium, and PRA levels were unchanged by atriopeptin. Hematocrit increased by the infusion of Atriopeptin

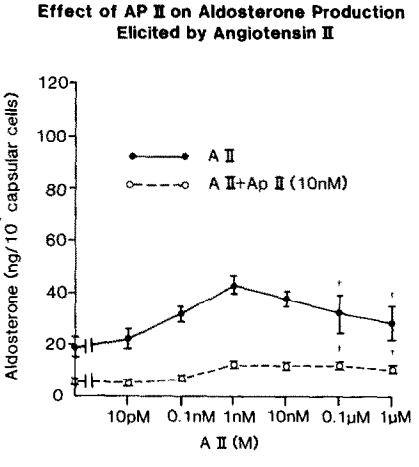


Fig. 2. Aldosterone production by Angiotensin II with or without Atriopeptin II. Each point represents a mean ± SE of 5 experiments done in duplicate, except where indicated by †, where $n = 2$. All concentrations are expressed as M/l of incubation medium.

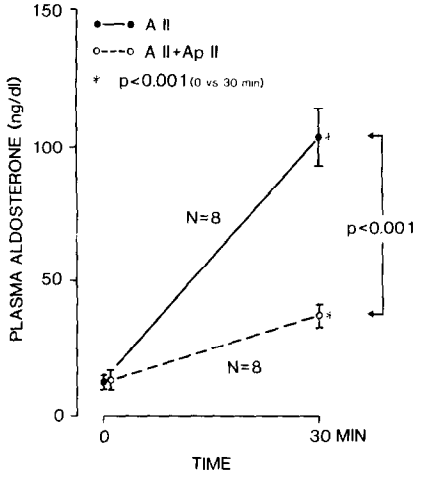


Fig. 3. *In vivo* inhibition by Atriopeptin II of plasma aldosterone stimulation by Angiotensin II. Angiotensin II is infused either alone or with Atriopeptin II. Plasma aldosterone is shown on the ordinate and the time of infusion on the abscissa. $n = 8$ means 8 rats in each group were studied. * = $P < 0.001$.

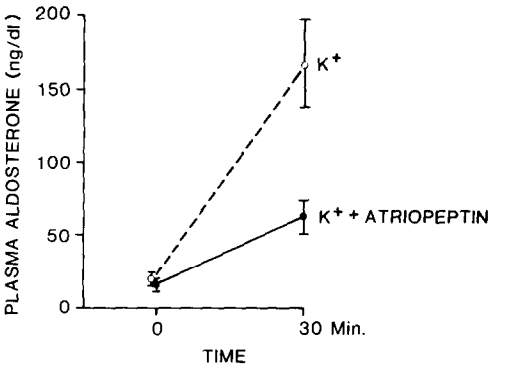


Fig. 4. Plasma aldosterone levels before and after infusion of 0.08 mEq/kg per min of potassium (●----●) or 0.08 mEq/kg per min of potassium with Atriopeptin II (●——●). Each point represents mean ± SE.

II, probably due to the diuresis and the increase in capillary permeability that occurs with atriopeptin infusions.

Figure 4 shows the effect of atriopeptin on the stimulation of aldosterone production by increasing the serum potassium concentration. Infusion of potassium chloride alone caused a marked increase in plasma aldosterone within 30 min along with a significant increase in the serum potassium level to 6.5 mEq/l. When Atriopeptin II was infused simultaneously, the aldosterone response to potassium was blunted even though the potassium concentration was not significantly different. Plasma renin activity and corticosterone levels were also similar in the two groups. It appeared, therefore, that the stimulation of aldosterone by potassium was blocked at the adrenal level.

The effect of infusion of Atriopeptin III on basal aldosterone production *in vivo* in rats maintained on a normal sodium diet is shown in Table 1. The rats were infused with either isotonic saline or atriopeptin III. The results of two different doses of atriopeptin III are shown in Table 1. Plasma aldosterone was measured before and at the end of the infusion. Plasma renin activity and electrolytes were measured at the end of the infusion. Plasma renin activity, corticosterone, sodium and potassium were measured at the end of the infusion. The control plasma aldosterone was 17 ng/dl. At the end of the saline infusion, the level was slightly decreased to 12 ng/dl. When the lower dose of Atriopeptin III was infused at $5 \times 10^{-11}$ mol/kg per min, the aldosterone levels and PRA were unchanged from the control. However, when a higher dose of atriopeptin was infused $5 \times 10^{-10}$ mol/kg per min there was a significant increase in PRA at the end of the infusion as well as an increase in corticosterone. Aldosterone increased from the pre-infusion levels of 17 ng/dl to 22 ng/dl, which is significantly different from the

Table 1. The low dose ANF did not affect PRA or plasma aldosterone. However, the high dose ANF significantly increased PRA and plasma aldosterone

| No.: | Saline alone 9 | Low dose ANF 9 | High dose ANF 7 |
|---|---|---|---|
| PA before infusion (ng/dl) | 17.3 ± 1.8 | 20.5 ± 3.4 | 17.0 ± 2.6 |
| PA after infusion (ng/dl) | 11.7 ± 0.5 | 12.4 ± 1.0 | 22.3 ± 1.4* |
| PRA after infusion (ng AI/ml per h) | 2.51 ± 0.37 | 3.43 ± 0.61 | 8.20 ± 1.48* |
| Plasma corticosterone (μg/dl) | 0.58 ± 0.07 | 1.56 ± 0.81 | 5.04 ± 1.22* |
| Plasma K (mEq/l) | 3.83 ± 0.08 | 3.86 ± 0.08 | 3.80 ± 0.10 |

Low dose ANF = bolus $5 \times 10^{-10}$ M/kg + $5 \times 10^{-11}$ M/kg per min for 30 min.
High dose ANF = bolus $5 \times 10^{-9}$ M/kg + $5 \times 10^{-10}$ M/kg per min for 30 min.
Values represent mean ±SE. * $P < 0.001$ compared with saline alone. Comparing saline alone with the low dose ANF there was no significant difference in any of the parameters. The high dose ANF significantly increased PRA, plasma aldosterone and corticosterone.

saline controls. In separate experiments, this higher dose of ANF reduced the blood pressure by 15–25 mm Hg and caused a marked diuresis. Presumably, this hypotension enhanced renin and ACTH release.

In Fig. 5 are shown the results of infusions of synthetic atrial natriuretic factor into hypertensive humans on a normal sodium diet. This figure shows a significant lowering of aldosterone and plasma renin activity during the infusion into 6 hypertensive subjects. The dose of ANF infused was 0.7 μg/min and caused only a slight decrease in systolic blood pressure and a mild diuresis without any change in plasma cortisol, serum sodium, potassium, ADH, or catecholamine levels.

*Calcium efflux*

The effect of superfusion of atriopeptin on calcium efflux was studied in isolated rat glomerulosa cells placed on a Sephadex G-15 column. When Angiotensin II was superfused over the cells there was a transient increase in efflux of $^{45}Ca$. This efflux was not significantly blocked by the addition of rat atriopeptin 1–28, despite the fact that this dose of ANF almost completely inhibited the stimulation of aldosterone secretion by angiotensin II (368 pg/$10^6$ cells in 2 min reduced to 22 pg/$10^6$ cells in 2 min).

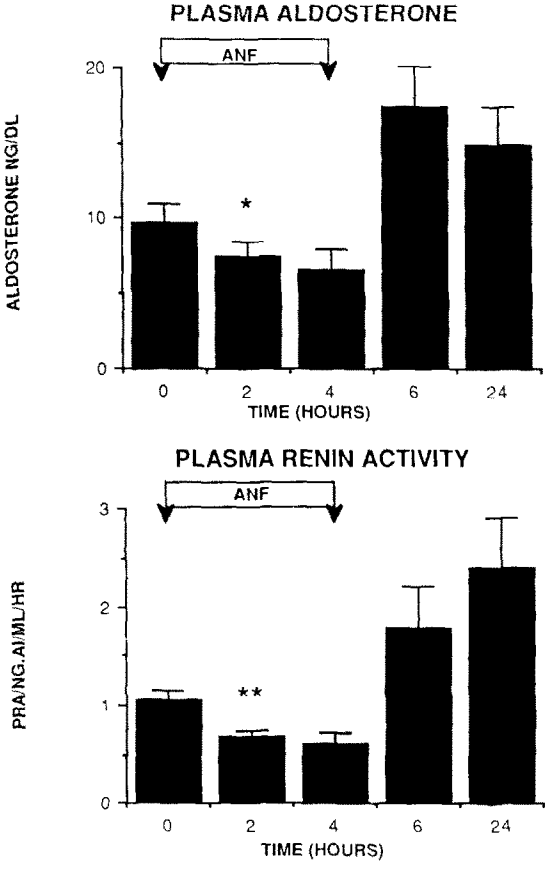


Fig. 5. Effect of a 4-h infusion of synthetic Met-ANF 8-33 (0.7 μg/min) upon plasma renin activity and aldosterone in 6 hypertensive patients. The arrows indicate the beginning and end of the infusion. The infusion began after the zero time blood was drawn. The infusion ended at 4 h. *Indicates significance <0.025 and ** significance <0.005.

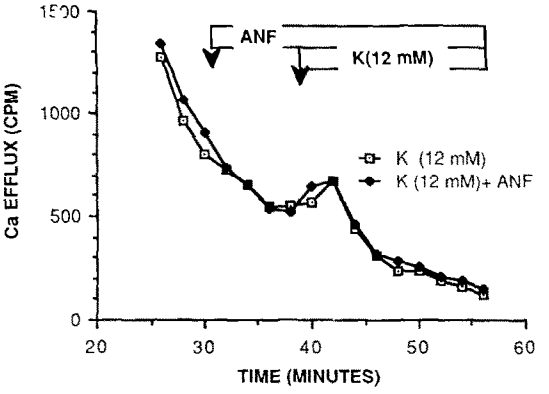


Fig. 6. Efflux of $^{45}Ca$ from superfused adrenal zona glomerulosa cells prelabelled with $^{45}Ca$. The first arrow indicates the start superfusion with media containing ANF $10^{-8}$ M and the second arrow indicates the superfusion with media containing both ANF and a high potassium concentration.

In Fig. 6 is shown a similar type of study done with cells superfused with high-potassium media. As can be seen, the rat adrenal glomerulosa cells pre-labeled with $^{45}Ca$ showed a significant increase in the efflux of $^{45}Ca$ when a high concentration of potassium was added to the media. When ANF (rat antriopeptin 1–28) was added to the superfusate before the addition of the high potassium concentration, there was no change in the calcium efflux curve. However, as shown in Fig. 7, the marked aldosterone stimulation by potassium was completely inhibited by ANF.

## DISCUSSION

The results reported in this manuscript, as well as those described in the literature, clearly demonstrate

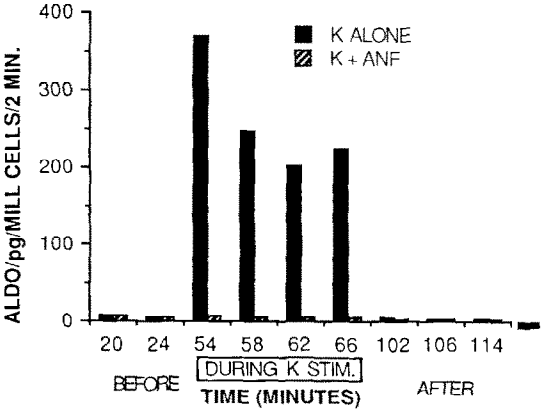


Fig. 7. Secretion of aldosterone during the superfusion of the zona glomerulosa cells in the same system for the $^{45}Ca$ efflux experiment. The black bars indicate superfusion with 12 mM K alone. The cross-hatched bars indicate cells superfused with 12 mM K plus $10^{-8}$ M ANF.

that atrial natriuretic factor inhibits aldosterone production, both basal and stimulated, by a direct action on the adrenal gland. This action of atrial natriuretic factor is specific for the zona glomerulosa. *In vivo* data also support a direct inhibition of aldosterone secretion. The stimulation of aldosterone secretion by infusions of Angiotensin II or potassium in the rat is inhibited by simultaneous infusions of atrial natriuretic factor. This effect seems to be independent of any action on plasma renin activity, serum sodium or potassium, or ACTH. It would appear, therefore, to be a direct action at the adrenal level. The failure of infusions of atriopeptin in the present experiments to inhibit basal aldosterone secretion in the rat needs explanation. *In vivo* studies are complicated by the fact that ANF in large doses can cause marked natriuresis and hypotension which in turn can set into play homeostatic mechanisms which increase plasma renin activity and ACTH release, both of which might offset the inhibitory effect of ANF on aldosterone production. In man, using a very low dose of ANF (approximately $3 \times 10^{-12}$ mol/kg per min), basal aldosterone secretion was reduced.

Reports in the literature on the effect of infusions of ANF on renin release are conflicting [12–20]. The results appear to depend upon the experimental condition. Therefore, *in vivo*, ANF may inhibit aldosterone production not only by direct action on the adrenal gland, but also by inhibition of renin release from the kidney.

The mechanism by which ANF inhibits aldosterone production at the adrenal level is not fully understood. There are specific plasma membrane receptors to ANF which are different from those of ACTH and Angiotensin II [21]. ANF has also been shown to inhibit adenyl cyclase in adrenal membranes, but this would not explain inhibition of the action of Angiotensin II. Also, ANF has been shown to stimulate cyclic GMP in rat adrenal glomerulosa cells [22]. However, the threshold dose for stimulating cyclic GMP is usually higher than the dose that inhibits aldosterone production. Furthermore, the addition of derivatives of cyclic GMP does not significantly inhibit aldosterone production *in vitro*. Nevertheless, the physiological significance of this effect of ANF on adrenal cyclic GMP generation needs to be better understood. Membrane receptor for ANF and the membrane guanyl cyclase enzyme are closely associated.

All stimuli such as ACTH, Angiotensin II, and potassium, enhance the production of a labile protein which, in turn, increases the entrance of cholesterol to the inner mitochondrial membrane where it is converted to pregnenolone. This step appears to be rate-limiting in steroidogenesis. Inhibitors of protein synthesis are known to hinder the action of ACTH, Angiotensin II, or potassium. Studies by other investigators have shown that the early step, conversion of cholesterol to pregnenolone [23], and a later step conversion of corticosterone to aldosterone [24] are inhibited by ANF. However, the initial event with ANF is still a mystery. In order to understand how ANF can block all three stimuli, one would have to search for some factor that is an intermediate step in the stimulation of aldosterone production by three stimuli. One possibility may be calcium. Angiotensin stimulates the turnover of membrane phospholipids with the generation of inositol triphosphate ($IP_3$) which mobilizes intracellular calcium from endoplasmic reticulum to increase the concentration of free cytosolic calcium. However, Goodfriend found no affect of ANF on the turnover of phospholipids stimulated by angiotensin in bovine adrenal cells *in vitro* [23]. We studied this system in another way. When the glomerulosa cells are pre-labeled with $^{45}Ca$, an increase in free cytosolic calcium stimulated by Angiotensin II leads to a transient increase in the efflux of $^{45}Ca$. Substances which can block intracellular mobilization of calcium will block the efflux of $^{45}Ca$ and reduce the response of aldosterone to Angiotensin II [10]. However, in our efflux experiment we were unable to show any significant inhibition of calcium efflux despite the fact that aldosterone production was markedly inhibited by ANF. More recently, Koijima *et al.*[25] emphasized a critical role of calcium influx in response to ACTH, Angiotensin II, and potassium. They argue that the critical feature to all three stimuli is the cycling of calcium across the membrane resulting in activation of enzymes that are required for the sustained aldosterone response to the stimuli. It is clear from previous studies that the initial effect of potassium stimulation involves the depolarization of the cell membrane with an influx of extracellular calcium and a transient increase in cellular calcium concentration. This increase is due to influx and not to mobilization of intracellular calcium. We therefore studied the effect of ANF on the efflux of calcium since the efflux would depend upon influx. In contrast to what one finds with calcium channel blockers, ANF does not inhibit the efflux and presumably, therefore, the influx of calcium stimulated by increasing the potassium concentration in the media. Nevertheless, ANF did markedly inhibit aldosterone production by the increased potassium. Thus, it appeared that blocking calcium influx is not a mechanism by which ANF inhibits all three stimuli of aldosterone production. One must, therefore, look for some effect beyond this action of the stimuli.

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