CHANGES IN PLASMA ALDOSTERONE FOLLOWING
THE ADMINISTRATION OF VARIOUS COMBINATIONS OF STIMULI

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ABSTRACT

The effects on plasma aldosterone levels of combinations of the main factors involved in aldosterone regulation have been studied in normal young male subjects. These factors, namely K, angiotensin II and ACTH were studied using combinations of K+ with angiotensin II, K+ with ACTH, and angiotensin II with ACTH.

KCl infused in amounts (5–10 mEq/h) insufficient to increase plasma aldosterone by itself was found to potentiate the aldosterone response to an infusion of angiotensin II (7 ng/kg/min) in 8 out of 13 subjects. The same amount of KCl was also found to increase the rise in plasma aldosterone following an infusion of ACTH (16 ng/kg/min).

When an angiotensin II infusion was superimposed on the last 2 h of a 4-h infusion of ACTH, a striking rise in plasma aldosterone was observed, which exceeded the values obtained by ACTH alone. Conversely, when an ACTH infusion was superimposed on the last 2 h of a 4-h angiotensin II infusion, a further increase of plasma aldosterone was again observed.

Our data support the idea that changes in all the known stimuli of aldosterone secretion have to be taken into account when interpreting changes in plasma aldosterone. Thus KCl, infused at rates without effect on aldosterone when given alone, will increase aldosterone during angiotensin II or ACTH infusion. The data also demonstrate that no refractory period is observed when one stimulus is superimposed on a previous one.

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We have studied in man the combined effect of three factors regulating the secretion rate of aldosterone, namely angiotensin II, ACTH and potassium. Angiotensin II and ACTH were administered in doses large enough to stimulate aldosterone secretion, while K\(^+\) was given in amounts insufficient to increase plasma aldosterone by itself.

\textit{In vivo} and \textit{in vitro} evidence points to an important role of K\(^+\) as a regulator of aldosterone secretion, this ion appearing to act directly on steroid biosynthesis in the adrenal gland (Boyd \textit{et al}. 1971; Boyd \& Mulrow 1972; Baumber \textit{et al}. 1971). In a previous study (Birkhäuser \textit{et al}. 1973) we showed that minute amounts of KCl, which did not modify plasma K\(^+\), could increase plasma aldosterone above the plateau obtained during a constant angiotensin II infusion. We had postulated that changes in intracellular K\(^+\) at the level of the adrenal, rather than changes in plasma K\(^+\) concentration, exerted the regulatory function in aldosterone biosynthesis. These experiments have now been repeated in a large group of subjects, and we have studied the effect of these small amounts of K\(^+\) on aldosterone secretion already stimulated by ACTH. We have also studied the effects of superimposing an angiotensin II infusion on an established continuous stimulation by ACTH (and \textit{vice versa}) to determine whether the combination would have an additive effect, or whether there would be a relative refractoriness to stimulation.

Finally, as the literature is not in agreement about the relationship between endogenous ACTH and the response of aldosterone to angiotensin II (Lieberman \& Luetscher 1960; Ross \textit{et al}. 1960; Williams \textit{et al}. 1971), we investigated the plasma aldosterone response to angiotensin II in subjects whose endogenous ACTH had been suppressed by dexamethasone.

**MATERIALS AND METHODS**

The experiments were performed on healthy male medical students (age range 23 to 30 years) from whom informed consent was obtained. The subjects, who were on an \textit{ad libitum} diet, were fasted overnight, and then remained recumbent during the experimental period which started between 7 and 8 a.m. and lasted for 4 h. Infusions and blood sampling were performed as previously described (Birkhäuser \textit{et al}. 1973).

Plasma renin activity (PRA) was determined by radioimmunoassay (RIA) (Poulsen \& Jørgensen 1974; Vallotton 1971) and plasma cortisol by a competitive protein binding method (Lecterq \textit{et al}. 1969). Plasma aldosterone was determined by the double isotope derivative dilution method (Scholer \textit{et al}. 1972) or by RIA (Underwood \& Williams 1972). The 2 methods were previously compared by Gaillard \textit{et al}. (1976). Standard methods were used for the determination of electrolytes, haematocrit, haemoglobin, proteins and osmolality.

The blood pressure was measured by sphygmomanometer at regular intervals throughout the angiotensin II infusions.

The data were examined using the non-parametric Wilcoxon test or the paired Student's \textit{t}-test to assess the statistical significance of the results, which are expressed as mean ± SEM, except when noted.
Protocols

The solutions to be infused were prepared in 5% glucose and the rate of infusions varied between 50 and 80 ml/h for angiotensin II, ACTH and dexamethasone, and was 250 ml/h for K+. The rate of substance infused was 7 ng/kg/min for angiotensin II (Hypertensin®, Ciba) 16 ng/kg/min for ACTH (Synacthen®, Ciba), except in protocol II (see below), 1 mg/h for dexamethasone (Decadron®, Merck, Sharp & Dohme) and 5 or 10 mEq/h for KCl. The infusions lasted for 2 or 4 h.

The protocols used were the following:

Protocol I. – Effect of potassium

1. Potassium alone
   a. Infusion of 5 mEq/h KCl from 120 to 240 min (n = 3).
   b. Infusion of 10 mEq/h KCl from 0 to 240 min (n = 4).

2. Potassium and angiotensin II
   a. Angiotensin II infusion from 0 to 240 min (n = 4).
   b. Angiotensin II infusion from 0 to 240 min and KCl infusion from 120 to 240 min (5 mEq/h KCl, n = 8; 10 mEq/h KCl, n = 5).

3. Potassium and ACTH
   a. ACTH infusion from 0 to 240 min (n = 6).
   b. ACTH and KCl (10 mEq/h) infusion from 0 to 240 min (n = 4). ACTH was infused after a priming dose of 4 μg/kg body weight according to the protocol of Cenac et al. (1975) to achieve plateau levels of plasma aldosterone more rapidly.

Protocol II. – ACTH and angiotensin II

a. ACTH infusion from 0 to 240 min and angiotensin II infusion from 120 to 240 min (n = 2). Priming dose of ACTH as in protocol I.
   b. Angiotensin II infusion from 0 to 240 min, and ACTH infusion from 120 to 240 min at a rate of 0.1 mg/h. This rate corresponded to a dose of 7 ng/kg/min for one subject and 4 ng/kg/min for the other (n = 2).

Protocol III. – Angiotensin II and dexamethasone

a. Angiotensin II infusion from 0 to 120 min (n = 6).
   b. Angiotensin II and dexamethasone infusion from 0 to 120 min (n = 6). Two mg dexamethasone (Millicorten®, Ciba) was administered at midnight on the previous day.

RESULTS

No changes in haematocrit, haemoglobin, proteins and plasma Na+ were observed in any of the experiments; therefore, volume changes, if any, were minimal. During angiotensin II infusion, endogenous plasma renin activity was suppressed to levels at the limit of detectability, and diastolic blood pressure was increased by 15–20 mmHg in all subjects.
Protocol I. – Effect of potassium

1. Potassium alone (Fig. 1)
   Plasma K\(^+\) did not change when K\(^+\) was infused at 5 mEq./h but increased from 4.12 ± 0.11 at 0 min to 4.55 ± 0.10 at 240 min when infused at 10 mEq./h.
   In these 2 groups, the plasma aldosterone values were not statistically different from the basal values of the control group observed during the same period.

2. Potassium and angiotensin II (Fig. 2)
   a. In the group receiving angiotensin II alone, plasma aldosterone increased rapidly from 5.3 ± 1.5 ng/100 ml to a mean plateau value of 20.9 ± 1.2 ng/100 ml, which was maintained from 60 to 240 min. Plasma K\(^+\) did not change.
   b. In the group receiving angiotensin II and KCl, plasma aldosterone rose from a basal value of 7.3 ± 1.0 ng/100 ml to a mean plateau value of 21.7 ± 1.1 ng/100 ml during the first 2 h of angiotensin II infusion. During the KCl infusion, plasma aldosterone increased further in 4 out of 8 subjects receiving 5 mEq. K\(^+\)/h and in 4 out of 5 subjects receiving 10 mEq. K\(^+\)/h to a mean value of 34.4 ± 2.5 ng/100 ml at 240 min.
   The criterion for a response in plasma aldosterone was a further increase of

Fig. 1.
Plasma aldosterone values during KCl infusion.
Upper part: 5 mEq./h for 2 h (n = 3). Lower part: 10 mEq./h for 4 h (n = 4). Shaded area: mean plasma aldosterone values (± sd) from 8 to 12 a.m. in 7 control subjects.
Upper part: effect on plasma aldosterone of a constant angiotensin II infusion (7 ng/kg/min, n = 4) from 0 to 240 min.

Lower part: effect on plasma aldosterone of the same angiotensin II infusion but combined with KCl infusion (5 or 10 mEq/h) for the last 2 h (n = 13). Upper line (n = 8) shows the responders, the bottom line (n = 5) the non-responders. *P < 0.02 **P < 0.01.

more than 5 ng/100 ml from the highest plateau value. In the 8 responders the plasma aldosterone increment was found to be independent of the amount of KCl infused, and so the plasma aldosterone values from the 2 infusion rates have been pooled. The plasma aldosterone increase was statistically significant (P < 0.02 and P < 0.01 from 180 min to 240 min) when compared with the values obtained during angiotensin II infusion alone. The values obtained in the 5 non-responders have been pooled in the same way and were not statistically different from the values obtained in the group given angiotensin alone, nor from the plateau obtained during the first 2 h of the infusion. Plasma K⁺ did not increase during KCl infusion and was always higher in the responders compared with the non-responders. It was, respectively, 4.22 ± 0.06 compared with 3.88 ± 0.09 mEq./l at 0 min, and 4.24 ± 0.08 compared with 4.00 ± 0.10 mEq./l at 240 min.
3. Potassium and ACTH (Fig. 3)

a. In the group receiving ACTH alone, plasma K+ did not change significantly (4.00 ± 0.17 at 0 min and 4.03 ± 0.05 at 240 min). Plasma renin activity did not show any significant changes and plasma cortisol increased from 16.7 ± 2.0 to 34.5 ± 3.7 µg/100 ml at 240 min. Plasma aldosterone increased from 11.1 ± 1.1 ng/100 ml to a maximum of 20.4 ± 2.6 ng/100 ml at 60 min and decreased to 9.5 ± 1.2 ng/100 ml at 240 min.

b. In the group receiving ACTH and KCl, plasma K+ increased significantly from 3.97 ± 0.11 mEq./l at 0 min to 4.55 ± 0.10 mEq./l at 240 min (P < 0.01). Plasma renin activity did not change and plasma cortisol rose from 17.8 ± 2.5

![Fig. 3](image)

Effect on plasma cortisol, renin activity and aldosterone of an ACTH infusion (16 ng/kg/min) alone (n = 6) or combined with a KCl infusion (10 mEq./h, n = 4). In both experiments a priming dose of 4 µg/kg body weight ACTH was administered.

*P < 0.05  **P < 0.02  ***P < 0.01.
Effect on plasma cortisol, renin activity and aldosterone of superimposed infusions of angiotensin II (AII) or ACTH on a constant infusion of ACTH or angiotensin II (n = 2). The lines are individual subject data.

Protocol II. – ACTH and angiotensin II (Fig. 4)

a. In the 2 subjects receiving ACTH for 4 h, plasma K+ did not change, plasma cortisol rose steadily from 12.4 and 11.0 μg/100 ml at 0 min to 30.0 and 29.1 μg/100 ml at 240 min respectively. With ACTH alone, plasma aldosterone increased to a mean plateau value of 12.4 and 10.0 ng/100 ml in the 2 subjects. The superimposed angiotensin II infusion further increased plasma aldosterone to a maximum level of 39.7 and 21.6 ng/100 ml, respectively.

b. In the 2 subjects receiving angiotensin II for 4 h, plasma K+ did not change. Plasma cortisol declined gradually from 0 to 120 min and rose during the superimposed ACTH administration from 6.1 μg/100 ml in both subjects at 120 min to 22.1 and 29.3 μg/100 ml, respectively, at 240 min.

to 28.4 ± 3.8 μg/100 ml at 240 min. Plasma aldosterone increased from 8.8 ± 1.0 ng/100 ml at 0 min to 24.3 ± 4.5 ng/100 ml at 60 min and to 17.1 ± 1.4 ng/100 ml at 240 min. The last 3 values at 210, 225 and 240 min are statistically higher than in the group receiving ACTH without K+ (P, respectively, < 0.05; < 0.02; < 0.01).
Plasma aldosterone increased from 0 to 120 min to a mean plateau value of 13.0 and 17.1 ng/100 ml and increased further to a maximum level of 19.1 and 28.6 ng/100 ml in both subjects, respectively, during the superimposed ACTH infusion.

**DISCUSSION**

This paper reports the effects on plasma aldosterone of the combined administration of the main factors involved in aldosterone regulation, namely, K⁺ with angiotensin II or ACTH, and angiotensin II with ACTH.

Firstly, it was shown that the small amounts of KCl when infused alone did not alter plasma aldosterone. When given in the same amount during an infusion of angiotensin II, K⁺ produced an increase of plasma aldosterone above the level produced by angiotensin II alone. In some subjects, however, this increase was not observed; probably the amount of KCl used was at the threshold level. During ACTH infusion alone, plasma aldosterone values did not reach a plateau value, but rose initially and then fell towards the control values. This decrease has been explained by a redistribution of the steroid primarily into the red cells when the increase in cortisol concentration displaces aldosterone from high affinity binding sites in plasma (Zipser et al. 1976). When K⁺ was infused concomitantly with ACTH, the decrease of plasma aldosterone was statistically smaller than in the group with ACTH alone. This stimulatory effect of K⁺ is probably due to a synergism of ACTH and K⁺, as the same amount of K⁺ alone was not able to alter plasma aldosterone concentration. However since plasma K⁺ did rise by 0.58 mEq./l an even greater effect on plasma aldosterone may have been expected. Similarly, during long-term experiments in man Williams et al. (1970) found that increasing K⁺ intake could enhance the secretion of aldosterone in response to ACTH.
When we successively applied 2 acute stimuli – namely angiotensin II and ACTH, both in doses sufficient by themselves to produce stimulation of the adrenal cortex – we found that the second stimulus immediately produced a further increase of plasma aldosterone. We did not observe a period of relative refractoriness for aldosterone secretion as suggested by Katz et al. (1972). These investigators observed that if a second stimulus is superimposed rapidly after a first one, it does not produce its expected effect on aldosterone.

It is generally recognised that aldosterone secretion is less dependent upon pituitary control than cortisol secretion. Nevertheless some experiments performed in animals as well as in man have demonstrated that, in the absence of ACTH, there is both diminished secretion and excretion of aldosterone as well as a diminished response to stimuli such as angiotensin II (Biglieri & Ganong 1961; Lieberman & Luetscher 1960; Rayyis & Horton 1971; Ross et al. 1960). In our study, abrupt ACTH inhibition by dexamethasone did not influence the response of plasma aldosterone to angiotensin II. Our results are in agreement with those of Williams et al. (1971) who observed that patients treated with glucocorticoids for many years have a normal aldosterone response to sodium depletion and to ACTH. It seems that the pituitary factor necessary for a normal aldosterone response is not ACTH.

In conclusion, our findings indicate that in acute studies in man, superimposing a known stimulus to aldosterone secretion on a pre-existing one causes an immediate further increase of plasma aldosterone. In the case of K⁺, a minute amount, itself insufficient to produce a measurable rise of plasma aldosterone, is capable of increasing the response to angiotensin II and to ACTH. In both cases synergism seems to have taken place.

ACKNOWLEDGMENTS

The authors are thankful to Mrs Ch. Ryser, R. Petkova, and M. Gourjon for technical, Miss V. Nicolet for secretarial help, Dr G. Williams for stylistic corrections and Prof. A. F. Muller for continuous encouragement and support.

This work was supported by the Swiss National Science Foundation (Grants No. 3.230-0.74 and No. 3.845-0.77) and the Bickel-Birkigt Foundation.

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Received on December 13th, 1978.