Effect of single and combined infusions of angiotensin II and aldosterone on colonic potential difference, blood pressure and renal function, in patients with adrenal deficiency

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Summary
1. The effect of single and combined infusions of angiotensin and aldosterone on colonic potential difference, blood pressure and renal function was studied in two normal male subjects and four female patients with adrenal deficiency maintained only on cortisone.

2. Aldosterone had its usual effect on colonic potential difference and it was possible to show that angiotensin had a small but definite effect of its own in the absence of aldosterone. The two hormones produced a summation response when given together.

3. The effects on renal function in two normal young male subjects were similar to those known previously. The response of the patients was different and probably reflected a number of factors, such as age, sex and long-standing adrenal deficiency.

4. Although the numbers were small, both normal subjects and patients showed a significantly greater rise of blood pressure with combined infusions of angiotensin and aldosterone than with angiotensin alone. The plasma concentrations of angiotensin were similar with both types of infusion, and so increased sensitivity to angiotensin in the presence of aldosterone is postulated.

Key words: aldosterone, angiotensin, adrenal deficiency, blood pressure, colonic potential difference, renal electrolyte excretion.

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Introduction
Colonic potential difference (CPD) is closely related to active sodium absorption from the colonic mucosa, and is strongly influenced by aldosterone both in animals and man (Cooperstein & Brockman, 1959; Edmonds & Marriott, 1968; Edmonds & Godfrey, 1970; Archampong & Edmonds, 1972), being increased after administration of aldosterone or after dietary sodium restriction, and greatly diminished after adrenalectomy in animals (Edmonds & Marriott, 1967).

Recently the quantitative relationship of the CPD to aldosterone infusions was further studied in normal humans, and a highly positive correlation was shown between the change in CPD and rising plasma aldosterone concentrations (Efstratopoulos, Peart & Wilson, 1974).

Experience with the intestinal actions of angiotensin II itself has been limited. So far, its action has been studied on splanchnic blood flow in man (De Bono, Lee, Mottram, Pickering, Brown, Keen, Peart & Sanderson, 1963), and on sodium and fluid transport in the jejunum and colon of rats (Davies, Munday & Parsons, 1969, 1972; Munday, Parsons & Shaikh, 1970; Hornych, Meyer & Milliez, 1973). In some experiments, the single and combined actions of both aldosterone and angiotensin II were studied in adrenalectomized rats. A maximum response (increase in transport) was obtained only with the combination of both hormones.

It was of interest therefore to study the responses of the CPD to intravenous infusions of aldosterone with and without angiotensin, in patients with bi-
lateral adrenalectomy or Addison's disease. Concurrently, the effects of combined (aldosterone plus angiotensin II) infusions on blood pressure and renal function were studied.

Methods

Patients and normal subjects

Four female patients, aged 43, 45, 64 and 65 years, were studied. The two youngest had had Cushing's syndrome and bilateral adrenalectomy for adrenal hyperplasia had been done 3 and 8 years previously; the other two had been treated for Addison's disease and hypothyroidism for many years.

Two healthy male volunteers, aged 19 and 20 years, were studied as control subjects.

Both the patients and the normal volunteers received a detailed explanation of the study and consent was obtained from the Ethical Committee of the Hospital.

All the patients were in hospital throughout the duration of the study; the normal volunteers arrived early on the morning of each experimental day.

Before the experiments, free diet and sodium intake was allowed (except liquorice-containing foods and alcohol). None of the normal subjects was taking any medicament. All the patients were taking their usual maintenance dose of cortisone acetate (25-50 mg/day) but none was taking 9α-fluorocortisol or other medication for at least 1 week before the study started.

Experiments

In each patient or normal subject, a set of four experiments was carried out involving respectively intravenous infusion of: (a) dextrose in water (0.36 mol/l; 50 g/l) at the same rate as in the experiments proper; (b) aldosterone (22 pmol min⁻¹ kg⁻¹); (c) angiotensin II (7.6 pmol min⁻¹ kg⁻¹); (d) aldosterone plus angiotensin II (22 and 7.6 pmol min⁻¹ kg⁻¹ respectively).

Each of the above experiments was carried out on a separate day and in random order for each subject studied. Two days for recovery were allowed between each experiment. On the day of the experiment, the subjects voided urine on rising; the time was recorded but no sample saved. Subsequently they stayed recumbent and fasting throughout the experiment, rising only for micturition.

When the patients or subjects were recumbent, a constant intravenous infusion of sodium chloride (0.16 mol/l; 9 g/l) at about 3 ml/min was started and maintained for about 11 h in order to provide an adequate sodium load for the adrenal-deficient patients.

In addition to the constant sodium chloride (saline) infusion, tap water was given orally (300 ml initially and 150 ml hourly thereafter) to maintain diuresis.

Infusion of aldosterone and/or angiotensin II solution, or the equivalent volume of dextrose in water (0.36 mol/l; 50 g/l) was given from a sterile syringe, driven by an infusion pump (Palmer, England), into the tubing of the saline infusion; each drug infusion lasted 2 h, starting 3 h after recumbency.

The first urine collection was completed 3 h from the start of the experiment, and subsequently 2-hourly specimens were taken until the end of the study. Venous blood samples (40 ml) were taken through a polyethylene cannula (Argyle Medicut, 18 GA), inserted beforehand into the opposite arm to that infused; the first sample was taken immediately before the infusion of aldosterone and/or angiotensin started, a second sample just before the end of hormone infusion and a final one 4 h after the end of infusion.

Plasma concentrations of sodium, potassium, creatinine, aldosterone, angiotensin II and osmolality were measured, as were urine concentrations of sodium and potassium.

Blood pressure was measured with a mercury sphygmomanometer every 2 min for the first half of angiotensin and/or aldosterone infusion, every 10 min for the second half and every hour before and after the hormonal infusion; the mean of three or four readings was recorded.

Colonic potential difference was measured just before the infusion of aldosterone and/or angiotensin and thereafter every 2 h until the end of the experiment, and again the next day about 18 h from the end of the infusion.

Solutions

Aldosterone. Aldocorten (Ciba) was diluted in 250 ml of dextrose in water (0.36 mol/l; 50 g/l), giving
a concentration of 5.5 μmol/l (2 μg/ml); it was given intravenously to each subject over 2 h at a rate of 22 pmol min⁻¹ kg⁻¹ (8 ng min⁻¹ kg⁻¹).

**Angiotensin.** Val⁵ angiotensin II amide ( Hypertensin, Ciba) was diluted in 250 ml of dextrose in water (0.36 mol/l; 50 g/l), giving a concentration of 1.9 μmol/l (2 μg/ml), which was infused intravenously over 2 h in each person at a rate of 7.6 pmol min⁻¹ kg⁻¹ (8 ng min⁻¹ kg⁻¹).

**Aldosterone plus angiotensin.** The same weight of each of the substances was made up in the same volume of dextrose in water and infused intravenously to give the same dose rates as in the separate infusions previously.

**Expression of results**

Where appropriate results have been expressed as mean value ± SEM.

**Results**

**Effect of infusion of angiotensin and/or aldosterone upon the corresponding plasma concentrations**

The increase of plasma angiotensin concentrations observed during the angiotensin infusion, or in

![Graph showing plasma angiotensin II levels](image)

**FIG. 1.** Plasma angiotensin II during infusion of (a) angiotensin alone (7.6 pmol min⁻¹ kg⁻¹) and (b) angiotensin with aldosterone (7.6 and 22 pmol min⁻¹ kg⁻¹ respectively) in the patients (●) and the normal subjects (○). Horizontal bars denote the infusion period.

**TABLE 1. Effect of intravenous infusion of aldosterone and/or angiotensin II (22 and 7.6 pmol min⁻¹ kg⁻¹ respectively) upon plasma aldosterone concentration in two normal male subjects**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Angiotensin II infusion</th>
<th>Aldosterone infusion</th>
<th>Angiotensin II + aldosterone infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min 120 min</td>
<td>0 min 120 min</td>
<td>0 min 120 min</td>
</tr>
<tr>
<td>C.W.</td>
<td>11-1 23-5 13-9 155-4 11-1 191-0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T.S.</td>
<td>11-1 22-2 8-3 108-2 2-78 133-2</td>
<td></td>
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</tbody>
</table>
combination with aldosterone, is shown in Fig. 1; the increase of plasma angiotensin during the infusion of angiotensin in both the patients and the normal subjects was 224 ± 11·1 pmol/l (236 ± 11·7 pg/ml) and during the combined infusion it was 253 ± 14·0 pmol/l (266 ± 14·7 pg/ml), which was not significantly different (0·1 < \( P < 0·2 \)).

Table 1 shows the plasma aldosterone concentrations observed during the single and combined infusions of the two hormones, in the two normal subjects studied. There is no great difference between the levels achieved with aldosterone or angiotensin plus aldosterone. These are similar to the normal responses observed previously (Efstratopoulos et al., 1974).

![Graph showing effect on colonic potential difference (CPD) of infusions of angiotensin, aldosterone, and angiotensin plus aldosterone in four patients with bilateral adrenalectomy or Addison's disease.](image)

**Fig. 2.** Effect on colonic potential difference (CPD) of infusions of angiotensin (Δ), aldosterone (○) and angiotensin plus aldosterone (▲) in four patients with bilateral adrenalectomy or Addison's disease. ○, Control experiments. Values given (mean ± SEM) represent the difference (Δ) from the base-line value measured just before the infusion started.

**Response of colonic potential difference to intravenous infusion of aldosterone and/or angiotensin**

During control experiments (0·3 mol/l dextrose infusion), CPD was practically unchanged in both the patients and the normal subjects (Fig. 2).

The intravenous infusion of angiotensin caused an increase in the electronegativity of colonic mucosa CPD by 7–9 mV with a mean value of 8 ± 0·9, which was the same for the patients and the control subjects; the time-course of change in CPD was the same in all experiments with a peak response 4 h from the end of the infusion (Fig. 2, which shows only the patients' responses).

Infusions of aldosterone in the adrenalectomized and Addisonian patients gave the same CPD response as we have previously observed in normal subjects (Efstratopoulos et al., 1974); for these patients the maximum change of CPD was 24·3 ± 1·4 mV, 4 h from the end of the infusion (Fig. 2).

The combined infusion of aldosterone and angiotensin produced a greater mean CPD response (34 ± 2·7 mV) than the aldosterone infusion, the time of peak response being the same (Fig. 2). After reaching its peak 4 h from the end of the infusion, the CPD returned to base-line values at 18 h from the end of the infusion.

The highest maximum response of CPD observed during the combined infusion of aldosterone and angiotensin was significantly different from that observed during the single aldosterone infusions (0·01 < \( P < 0·02 \)). The maximum rise of CPD during combined aldosterone plus angiotensin infusions was very similar to the sum of maximum CPD rises obtained during the single infusions of aldosterone and angiotensin (Fig. 2), suggesting that the increased response of colonic mucosa to the double stimulus of aldosterone and angiotensin was not due to an increased sensitivity to the action of aldosterone in the presence of high plasma angiotensin concentrations, but was a summation effect.

**Effect of angiotensin and/or aldosterone infusions on blood pressure**

The results of eighteen experiments with intravenous infusion of angiotensin and/or aldosterone, in both the patients and the normal subjects, are set out in Table 2 (see also Fig. 3). It is apparent that:

(a) aldosterone infusion had very slight or no effect on mean blood pressure, (b) angiotensin infusion produced a distinct increase (19 ± 1·2 mmHg) of mean blood pressure in the patients, whereas the two young normal volunteers showed smaller responses, and (c) the combined angiotensin plus aldosterone infusion gave a much greater increase of mean blood pressure than the single angiotensin infusion in both the patients and the normal subjects. During the combined infusion, the rise in mean blood pressure for the patients was 37 ± 4 mmHg. This value was significantly greater than the corresponding value with angiotensin alone (0·0125 < \( P < 0·025 \)).
Angiotensin on colonic potential difference

Fig. 3. Effect of infusion of dextrose (○), angiotensin (●), aldosterone (△) and of angiotensin plus aldosterone (▲) on the mean blood pressure in one of the patients with adrenal deficiency.

Table 2. Maximum rise of mean blood pressure during intravenous infusions of angiotensin II and/or aldosterone in the patients and normal subjects

<table>
<thead>
<tr>
<th></th>
<th>Maximum rise of mean B.P. (mmHg)</th>
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<tbody>
<tr>
<td></td>
<td>Aldosterone</td>
</tr>
<tr>
<td>Patients</td>
<td></td>
</tr>
<tr>
<td>J.F.</td>
<td>5</td>
</tr>
<tr>
<td>B.B.</td>
<td>6</td>
</tr>
<tr>
<td>M.B.</td>
<td>3</td>
</tr>
<tr>
<td>H.B.</td>
<td>6</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>19±1.2*</td>
</tr>
<tr>
<td>Subjects</td>
<td></td>
</tr>
<tr>
<td>T.S.</td>
<td>3</td>
</tr>
<tr>
<td>C.W.</td>
<td>4</td>
</tr>
</tbody>
</table>

The increased pressor response during the combined infusions was obtained almost immediately (about 2 min from the start of the infusion), just as during the single angiotensin infusions; after the first 15 min, the blood pressure fell to a lower value. When the infusion was stopped, the blood pressure quickly returned to base-line values or even lower (Fig. 3).

The increase in plasma angiotensin concentrations was similar in all recipients (Fig. 4), despite the different rises in mean blood pressure. It is obvious that the response of increased blood pressure to the combined infusion was not due to increased plasma levels of angiotensin but was related to the concurrent infusion of aldosterone, and is in favour of increased sensitivity to angiotensin due to the presence of elevated aldosterone concentrations.

Effect of aldosterone and/or angiotensin infusions on renal function

The results from the studies in the two normal subjects and the four patients are shown in Figs. 5(a) and
Fig. 5. Effect of dextrose, as control (○), and of angiotensin (●), aldosterone (■), and combined angiotensin plus aldosterone (▲) infusions on urine flow, sodium excretion (△U\textsubscript{Na}/V) and potassium excretion (△U\textsubscript{K}/V), (a) in normal subjects and (b) in the patients. Values shown are arithmetic means.

5(b). During the control infusions, normal subjects responded with a brisk natriuresis and potassium excretion and urine flow were little changed. The adrenal-deficient patients showed a progressive rise in urine flow accompanied by natriuresis, much less than in the normal subjects, with a delayed rise in potassium excretion. The effect of angiotensin in the normal subjects was to reduce the urine flow markedly and at the same time cause an anti-natriuresis and little change in potassium excretion. The addition of aldosterone to angiotensin did not change the reduction of urine volume but enhanced the anti-natriuretic effect of angiotensin. Aldosterone alone caused a marked anti-natriuresis, which persisted for many hours after the end of the infusion. In the patients neither the hormones singly nor in combination had much effect on urine flow until a decrease occurred 4–6 h after the end of the infusion. The anti-natriuresis due to angiotensin seen in the normal subjects was not apparent and even this effect with aldosterone was very transient. Potassium excretion rose during most of the infusions but was less with angiotensin. The major difference between the two groups of course was that the sodium excretory response during the control experiments in these patients was very much lower than in the normal subjects (Figs. 5a and 5b).

Discussion

It has been reported (Davies \textit{et al.}, 1969, 1972; Munday \textit{et al.}, 1970) that angiotensin II stimulates sodium and fluid transport in sacs of colon from rats previously subjected to bilateral adrenalectomy and nephrectomy (Crocker & Munday, 1967). Furthermore, although aldosterone is a major stimulus for
sodium and fluid transport through the colon, in both adrenalectomized and adrenalectomized plus nephrectomized rats, a maximum response is observed only in the presence of endogenous or added angiotensin (Munday et al., 1970).

Recently Hornych et al. (1973), studying the effects of angiotensin and cyclic-AMP in vitro in everted sacs of rat colonic mucosa, verified that angiotensin has a direct effect on sodium and water transport. These effects were apparent at physiological concentrations of the peptide, which are lower than those necessary for vasoconstriction and pressor action.

Transmucosal colonic potential difference is closely correlated to colonic sodium transport, which in turn is markedly affected by aldosterone. If angiotensin has an effect upon sodium transport through the colon in vivo, it might affect CPD. It has been shown (Edmonds & Marriott, 1967) that adrenalectomized, sodium-depleted rats maintained on cortisone had subnormal CPD values, which increased after aldosterone administration. The human equivalent in such studies is the patient with Addison's disease or bilateral adrenalectomy. In the four patients with adrenal deficiency maintained on cortisone acetate and sodium replete, and in the normal subjects, angiotensin infusion produced a moderate increase (7-9 mV) in the electronegativity of colonic mucosa. This response must therefore be independent of aldosterone.

Aldosterone infusion in the same patients gave a distinct increase of CPD (24±3 ±1±4 mV) similar to that observed with normal subjects (Efstratopoulos et al., 1974). The combined infusion of aldosterone and angiotensin caused a greater response of CPD than aldosterone alone and this increased response was not due to an increased sensitivity to aldosterone, but to an additive effect of the two hormones.

The peak response of CPD was obtained at the same time (i.e. the sixth hour from the start of the infusion) for each type of infusion used. There was no difference between patients and normal subjects in respect of the height and pattern of CPD change during the infusion studies.

A distinctive feature of the present study was the effect of combined hormonal infusion upon the blood pressure, especially in the patients. It has been suggested that untreated patients with Addison's disease have an increased 'threshold' of pressor response to angiotensin (i.e. the smallest infusion rate at which an effect is observed), and that after treatment with cortisol and fluorocortisol, plasma renin concentrations fall concurrently with 'threshold' (Chinn & Düsterdieck, 1972). This threshold change has also been correlated with the sodium balance, being increased in sodium-depleted subjects (Brown, Fraser, Lever & Robertson, 1971; Chinn & Düsterdieck, 1972). In our adrenal-deficient patients, maintained on cortisone and in normal sodium balance, combined infusion of aldosterone plus angiotensin produced a significantly greater increase of mean blood pressure than the angiotensin infusion alone. This greater response was immediate (see Fig. 3) and maintained throughout the infusion period. The increased blood pressure response was not associated with increased concentrations of angiotensin, showing that the presence of elevated plasma aldosterone concentrations increased in some way the pressor effects of angiotensin. The same effect on blood pressure was observed in the two normal young volunteers, although to a lesser degree. This observation might be relevant in patients with forms of hypertension where elevated plasma concentrations of both aldosterone and angiotensin exist.

The renal effects of angiotensin infusion in both normal subjects and Addisonian patients are well known (De Bono et al., 1963; Küchel, Horky, Kapitola & Motlik, 1964), and nothing remarkable emerged in the present studies.

Acknowledgments

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References


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