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EVIDENCE AGAINST AN INTERACTION OF ANGIOTENSIN II WITH THE SYMPATHETIC NERVOUS SYSTEM IN MAN

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SUMMARY

Animal experiments indicate that angiotensin II can, under some circumstances stimulate the sympathetic nervous system at a number of different sites. In order to determine whether such a relationship of the renin–angiotensin and sympathetic nervous system exists in man, we increased (by intravenous infusion), or decreased (by administering the oral converting enzyme inhibitor captopril) circulating angiotensin II levels and monitored plasma adrenaline and nor-adrenaline responses. Angiotensin II infusions did not increase plasma catechol-amines, and lowering of angiotensin II by captopril treatment in patients with severe hypertension or congestive heart failure failed to alter plasma adrenaline or nor-adrenaline levels. Whether physiological levels of angiotensin II are capable of interacting directly with the sympathetic nervous system in man remains to be demonstrated.

There is a vast literature attesting to interactions of the renin–angiotensin and sympathetic nervous systems (McCubbin, 1974). Whereas animal experiments leave no doubt that angiotensin II in large doses can stimulate the sympathetic system at multiple sites, it is less clear whether such an interaction occurs at physiological levels of angiotensin II in man.

In the present human studies we have increased or decreased plasma angiotensin II levels, and monitored catecholamine responses in plasma in an attempt to define whether angiotensin II is capable of altering sympathetic activity.

METHODS

The human studies were approved by the Human Use Committee of the University of

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Michigan Medical Center or the Ethical Committee of the North Canterbury Hospital Board, Christchurch, New Zealand.

Angiotensin II infusion in normal subjects

Five healthy male volunteers, aged 22–28 years, were studied in the Clinical Research Centre at the University of Michigan Medical Center. Each subject received a constant intake of dietary sodium (40 mmol/day) and potassium (100 mmol/day) for 4 days prior to angiotensin II infusions on day five. Smoking, caffeine-containing beverages, and vigorous physical exercise were avoided. On day 5 the volunteers remained supine in bed, and a venous cannula was inserted into either arm at 0800 h, one for infusion, the other for sampling. After 60 min of 5% dextrose administration at 0·2 ml/min, angiotensin II (Hypertensin, Ciba) was infused incrementally at 0·5, 1·0, 2·0 and 4·0 $ng.kg^{-1}.min^{-1}$, each rate for 1 h. Two venous samples were drawn during dextrose administration and single samples were obtained at the completion of each infusion rate of angiotensin II for measurement of nor-adrenaline, adrenaline and angiotensin II. Blood was taken into pre-chilled containers, immediately centrifuged at 4°C, and the plasma stored at −20°C until analysed. Blood pressure was measured at 10 min intervals using a standard mercury sphygmomanometer taking phase V as the diastolic endpoint: the mean of six recordings was taken as the blood pressure for each hour of infusion.

Blockade of angiotensin II formation in severe hypertension

Ten patients, nine male, one female, aged 28–53 years, five white, five black, with elevated blood pressure not controlled on conventional therapy were studied in the Clinical Research Center of the University of Michigan. Smoking and caffeine-containing drinks were avoided. In all patients, prior treatment consisting of propanolol (80 mg four times daily), hydrochlorothiazide (25 mg four times daily), and hydrallazine (50 mg four times daily) was discontinued on admission, and dose titration with the oral converting-enzyme inhibitor captopril (Squibb) started the following morning. With the patient supine in bed, captopril was administered at 2-hourly intervals in increasing dosage (25, 50, 100 and 150 mg) until a 'hypotensive' response (fall in diastolic pressure phase V of at least 10 mmHg using a conventional mercury sphygmomanometer) was achieved. A venous cannula was inserted 30 min prior to a baseline (pre-captopril) sampling, and a second venous specimen was obtained once the 'hypotensive' response was reached. Both samples were handled as described above and analysed for plasma angiotensin II, plasma renin activity (PRA), and plasma adrenaline and nor-adrenaline.

Blockade of angiotensin II formation in congestive heart failure

The oral converting enzyme inhibitor captopril, was administered to four male patients aged 62–72 years in the Intensive Care Unit, Princess Margaret Hospital, Christchurch, New Zealand. Details of haemodynamic and hormone responses have been reported (Maslowski *et al.*, 1981). Each patient was in severe congestive cardiac failure (grade III or IV New York Heart Association classification) and had proven resistant to conventional therapy. A diet of constant sodium (33–48 mmol/day) and potassium (73–100 mmol/day) was taken, and bedrest was enforced throughout the 7–10 day study. Digoxin (0·0625–0·5 mg/day) and frusemide (120–500 mg/day) therapy was constant in each patient for the duration of the study. After a 2-day 'run-in' period of digoxin and frusemide therapy, captopril was administered at 0730 h, 1430 h and 2330 h starting at 6·25 mg and increasing

until a maximum of 150 mg per dose or an intra-arterial systolic pressure of 75 mmHg was reached. Blood samples for measurement of PRA, angiotensin II, adrenaline and nor-adrenaline were drawn from an arterial catheter at 0830 h and 1530 h each day, precisely 1 h after captopril administration, and handled as described above.

Plasma angiotensin II (Nicholls & Espiner, 1976) and PRA (Dunn & Espiner, 1976) were measured by radioimmunoassay. The radioenzymatic technique used to measure catecholamines (Peuler & Johnson, 1977) was capable of detecting 2·5 pg/tube of adrenaline or nor-adrenaline added to plasma. There was a straight line relationship between catecholamines added to plasma and final counts per minute from the assay over a range of 10–1000 pg adrenaline or nor-adrenaline per tube. From fifteen consecutive assays the interassay coefficient of variation was 17% (adrenaline) and 11% (nor-adrenaline): the intra-assay coefficient of variation was 8% (adrenaline) and 7% (nor-adrenaline). All samples from any one subject were analysed in a single assay.

RESULTS

Angiotensin II infusion in normal subjects

Blood pressure did not change significantly until the highest infusion rate (4 $ng.kg^{-1}.min^{-1}$) was reached (Fig. 1) when the average increase in systolic pressure was 6 mmHg and in diastolic pressure was 9 mmHg above baseline. The pulse rate of 58·2 ± 3 beats/min (mean ± SEM) prior to angiotensin II administration was unaltered by infusion of the octapeptide (58·3 ± 3·4 beats/min during the highest infusion rate). Despite increments in plasma angiotensin II to approximately 100 pg/ml there was no clear response of adrenaline or nor-adrenaline (Fig. 1).

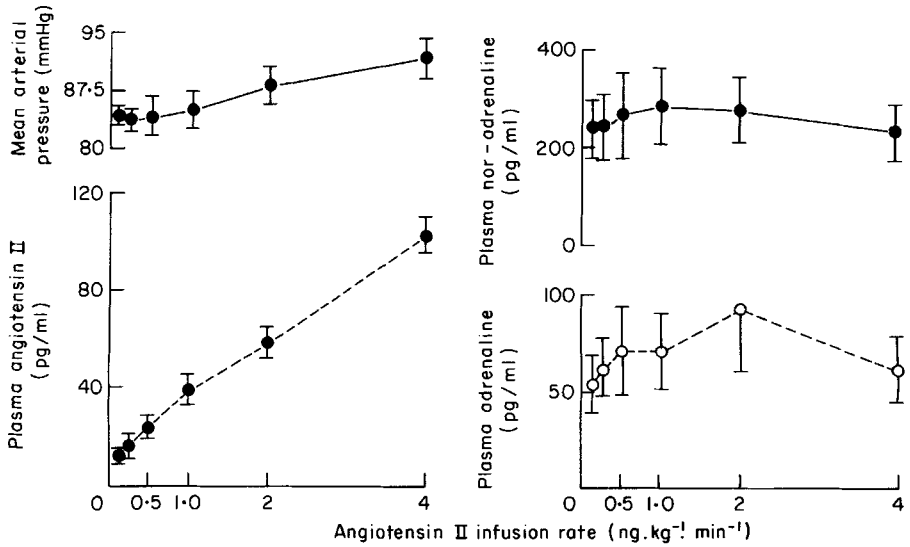

Fig. 1. Plasma hormone levels and arterial pressure (mean ± SEM) in five volunteers prior to and during an incremental intravenous infusion of angiotensin II. See Table 1 for conversion to SI units.

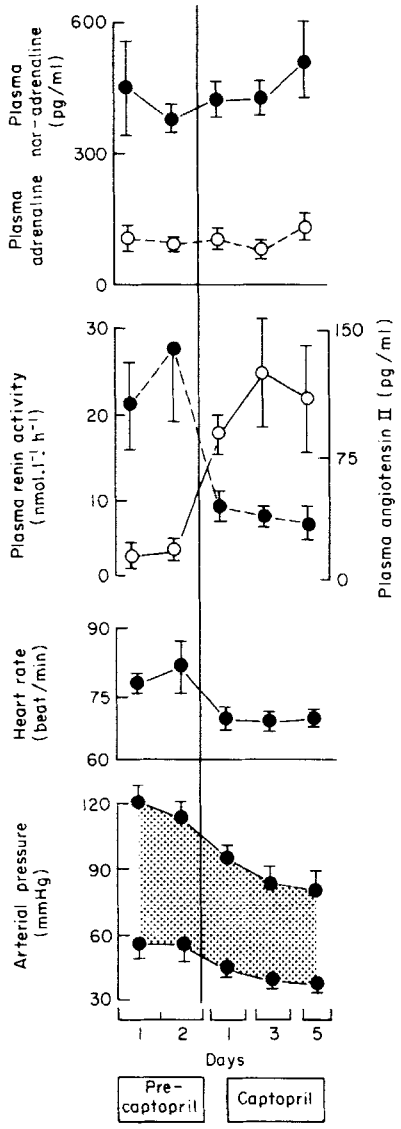

Fig. 2. Plasma hormone, arterial pressure and pulse rate responses to administration of the oral converting enzyme inhibitor captopril in four patients with congestive cardiac failure. Data are shown as mean ± SEM. See Table 1 for conversion to SI units. Plasma renin activity o——o; plasma angiotensin II •——•.

Blockade of angiotensin II formation in severe hypertension

Captopril treatment reduced angiotensin II levels in eight of ten patients, and PRA increased significantly (Table 1). On the contrary, plasma catecholamines were not altered for the group as a whole (Table 1), nor in the eight patients in whom a clear-cut decline in angiotensin II occurred (three showing either no change or a rise in adrenaline and nor-adrenaline levels).

Table 1. Plasma hormone concentrations (mean ± SEM) from ten hypertensive patients before and after captopril therapy

	Pre-captopril	Capropril
PRA ($ng.ml^{-1}.h^{-1}$)	1·61 ± 0·61	4·09* ± 1·62
Angiotensin II (pg/ml)	21·2 ± 3·2	12·5 ± 2·8
Nor-adrenaline (pg/ml)	177 ± 33	189 ± 37
Adrenaline (pg/ml)	57 ± 6	61 ± 9

*$P < 0·05$ significance of change from pre-captopril value (paired t test).

Conversion to SI units: PRA 1 $ng.ml^{-1}.h^{-1} = 0·77$ $nmol.^{-1}.h^{-1}$; angiotensin II 1 pg/ml = 0·95 pmol/l; nor-adrenaline 1 ng/ml = 5·91 nmol/l; adrenaline 1 ng/ml = 5·46 nmol/l.

Blockade of angiotensin II formation in congestive heart failure

Adrenaline and nor-adrenaline levels prior to administration of the oral converting-enzyme inhibitor were generally higher than values seen in healthy subjects. Captopril therapy induced clearcut decreases in plasma angiotensin II and increments in PRA, yet there was no fall in plasma catecholamines (Fig. 2). Pulse rates declined (Fig. 2) but the change failed to reach conventional levels of statistical significance. Arterial pressure was decreased by captopril therapy (Fig. 2).

DISCUSSION

There is no doubt that angiotensin II can alter the activity of the sympathetic nervous system under certain experimental circumstances (Campbell & Jackson, 1979; Peach, 1971; Yu & Dickinson, 1971). Most of the data, however, are derived from animal studies where the doses of angiotensin II have been large. Whether one can extrapolate from these highly experimental animal data to physiological circumstances in man, is not known.

Few workers have looked in detail at possible actions of angiotensin II on the sympathetic system in man, and what data are available appear contradictory. For example, McGrath *et al.* (1977) and Takishita *et al.* (1978) reported that the sympathetic nervous system was activated by the weak angiotensin agonist saralasin, whereas neither saralasin (Carey *et al.*, 1978; Vlachakis *et al.*, 1978) nor angiotensin II infusion (Mendelsohn *et al.*, 1980) altered plasma nor-adrenaline levels according to other authors. Likewise, blockade of angiotensin II formation with a converting enzyme inhibitor has been reported variously to increase (Heavey & Reid, 1978; Hulthen & Hokfelt, 1978) decrease (Curtiss *et al.*, 1978; Turini *et al.*, 1979), or have no effect (Bravo & Tarazi, 1979) on plasma nor-adrenaline levels in man under various circumstances.

In order to define any action of angiotensin II on the sympathetic nervous system, we increased angiotensin II by infusion, or decreased endogenous angiotensin II levels with a converting enzyme inhibitor, and measured circulating catecholamine responses. Our subjects were studied under carefully controlled circumstances with attention to details of

body posture, the avoidance of venepuncture just prior to sampling, and prohibition of sympathetic stimulation from caffeine or smoking. The assays used were capable of detecting minor changes in catecholamine and angiotensin II concentrations.

Under these conditions, we failed to observe any increase in plasma catecholamines in healthy volunteers during increments in plasma angiotensin II which were within physiological limits. Our results thus agree with the brief report by Mendelsohn *et al.* (1980) which indicated a lack of change in plasma nor-adrenaline levels across angiotensin II infusion in normal man. Whether greater increments in angiotensin II beyond those seen under normal circumstances can alter sympathetic activity in man, remains to be seen.

Conceivably, any action of angiotensin II on the sympathetic nervous system may be maximal at relatively low levels of circulating angiotensin II. If this were so, increments in angiotensin II might not further activate the sympathetic system, but a reduction of angiotensin II levels from normal or high values should result in a lowering of circulating catecholamines. The current studies indicate that blockade of angiotensin II formation did not regularly alter circulating nor-adrenaline or adrenaline. Baseline levels of angiotensin II were not high in our severe hypertensives, presumably because of prior beta-blocker therapy, and the reduction in plasma angiotensin II induced by captopril was therefore not great. In contrast, both angiotensin II and catecholamines were elevated in the patients with cardiac failure yet no change in circulating catecholamines was noted when angiotensin II levels exhibited a clearcut fall.

In summary, we have shown that neither increments nor decrements in circulating angiotensin II alter plasma catecholamine levels, the humoral markers of sympathetic activity, in man. It is tempting to conclude therefore that angiotensin II has little or no action on the sympathetic nervous system, at least under the conditions of the present studies. It is possible nevertheless, that any action of angiotensin II was in part obscured by alterations of arterial baroreceptor input to the sympathetic system by a concomitant rise (during angiotensin II infusion) or fall (with captopril therapy) in blood pressure. However no change in catecholamines occurred in our normal subjects during 0·5–2 $ng.kg^{-1}.min^{-1}$ angiotensin II infusion rates when little or no rise in arterial pressure had occurred. Likewise, captopril monotherapy reduced blood pressure in our hypertensives by little more than 10 mmHg, thus major baroreceptor stimulation would not be expected. It seems unlikely therefore that changes in blood pressure obscured an action of angiotensin II on the sympathetic nervous system.

A complicating issue in the interpretation of captopril studies is the fact that bradykinin levels would be expected to rise along with falls in angiotensin II. Since bradykinin, like angiotensin II has been reported to stimulate adrenal medullary secretion (Feldberg & Lewis, 1964) it is conceivable that a reduction in sympathetic stimulation from falling angiotensin II was counterbalanced by augmented stimulation by rising bradykinin levels. Whether there are in fact distinct increases in plasma bradykinin across captopril treatment is open to dispute, and refinements of current methodologies for the measurement of this labile peptide are required for clarification.

A final note of caution must be made regarding interpretation of the present results. There is some uncertainty whether plasma catecholamines reflect accurately the state of activity of the sympathetic nervous system under all circumstances. For example, Mancia *et al.* (1979) using a neck chamber technique showed that in normal subjects a reduction of baroreceptor activity had pressor effects without a concomitant change in plasma nor-adrenaline. Since the overspill of transmitter from sympathetic nerve endings into the

circulation is small, and because the clearance rate of nor-adrenaline released into plasma will almost certainly be altered under certain conditions, plasma levels of nor-adrenaline must be interpreted with caution.

The current studies in man fail to demonstrate any action of angiotensin II to alter plasma adrenaline or nor-adrenaline levels, the humoral markers of sympathetic activity. Whether angiotensin II at physiological concentrations is capable of interacting directly with the sympathetic nervous system in man remains to be demonstrated.

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