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EFFECT OF ANGIOTENSIN-CONVERTING ENZYME INHIBITOR ON RESPONSE OF PLASMA RENIN ACTIVITY AND ALDOSTERONE TO TILTING IN MAN

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1 The effect of an angiotensin-converting enzyme inhibitor (SQ 20,881; 0.5 mg/kg) on the response to tilting of plasma renin activity (PRA), plasma aldosterone and cortisol was studied in five normal subjects.

2 PRA rose significantly in both the supine and upright positions following administration of SQ 20,881; no significant effect on aldosterone or cortisol was observed.

Introduction

Tilting into the upright position causes an increase in plasma renin activity (PRA) and plasma aldosterone concentration (Gordon, Wolfe, Island & Liddle, 1966). It is believed that the sympathetic nervous system mediates the increase in renin activity and the raised renin leads to increased generation of angiotensin I from which two aminoacids are removed by converting enzyme to produce angiotensin II; the resultant rise in plasma angiotensin II is thought to be the stimulus to aldosterone secretion. An inhibitor of angiotensin converting enzyme has been developed (SQ 20,881; Collier, Robinson & Vane, 1973), and may find application in the diagnosis and management of certain patients with hypertension. The effect on the physiological responses of the renin-aldosterone system of interrupting production of angiotensin II has not, however, been studied, and we have therefore investigated the effect of the converting enzyme inhibitor on the response of PRA and aldosterone to tilting in normal man.

Methods

Studies were carried out in five healthy male volunteers aged from 20-21 and weighing between 56.3-74.0 kg. Each subject gave consent after full explanation of the nature of the investigation, and ethical approval was obtained from the Research Committee of St George's Hospital. Since the drug has had only limited use in man, each subject was examined physically before being accepted, and routine laboratory studies performed as in the previous investigation (Collier *et al.*, 1973). One subject developed erythema with itching and whealing over the vein immediately after injection of the drug; this faded in 80 minutes. No subject showed any change attributable to the drug in the physical examination and laboratory tests on the day following the experiment. Volunteers were not accepted if they were habitual smokers or took other drugs for any reason. Subjects were allowed only water for 12 h prior to starting the study, and neither food nor drink was allowed during the investigation.

Estimation of plasma renin activity

Samples of venous blood (10 ml) were taken and quickly transferred to chilled tubes containing EDTA. Samples were kept in ice until centrifugation at 4° C, and the plasma was stored below −15° C until assay. Plasma renin activity (PRA) was determined by radioimmunoassay using a commercially produced kit (Squibb Angiotensin I Immunotope) based on the method of Haber, Koerner, Page, Kliman & Purnode (1969). PRA was expressed as ng angiotensin I ml^{-1} plasma h^{-1} during incubation at 37° C. The within assay reproducibility of the method in this laboratory is ±7%; all samples from any one experiment were estimated in the same assay.

Estimation of plasma aldosterone

Plasma aldosterone was estimated by a radioimmunoassay according to the method of Ito, Woo, Haning & Horton (1972), using columns of sephadex LH20 for the initial chromatographic procedure.

Estimation of plasma cortisol

Plasma cortisol was measured by a fluorimetric method for 11-hydroxy-corticosteroids (Spencer-Peet, Daly & Smith, 1965).

Protocol for tilt studies

Subjects reported to the laboratory fasting, and rested supine on the tilt table. A catheter was introduced into an arm vein and kept patent by slow infusion of physiological saline (0.15 M NaCl). A cuff was placed on the opposite arm for recording arterial pressure by a standard sphygmomanometer; heart rate was measured from the electrocardiogram. In four of the five studies in which SQ 20,881 was to be given, the blood pressure response to bolus injections of angiotensin I (Schwartz-Mann, batch number GEN-700-H/Y J3) was assessed. After a minimum of 83 min rest supine and at least 25 min after the last injection of angiotensin I, an initial control venous sample was taken for estimation of PRA, aldosterone and cortisol. A second control sample was taken 30 min later, and the subject was then tilted to an angle of approximately 60°; further blood samples were taken after 30 and 60 min in the upright position, and blood pressure and heart rate were measured just prior to sampling. Subjects who felt faint during tilting were returned to the horizontal for a minute or so, and it was then possible to tilt them again. After 60 min in the upright position, the subject was returned to the horizontal and blood samples were taken after 60 and 90 minutes. SQ 20,881 was then infused intravenously in a dose of 0.5 mg/kg over a 2 min period. Further blood samples were taken after 30 and 60 min and the subject was then again tilted to 60°. Sampling was carried out as in the first tilt. At the conclusion of experiments using SQ 20,881, a further bolus of angiotensin I was given with the subject in the supine position to assess the degree of blockade.

In the first two studies using SQ 20,881, the blood withdrawn at sampling (which totalled approximately 200 ml) was immediately replaced with an equal volume of saline. In subsequent studies the subjects received during the second half of the investigation an infusion of their own blood (withdrawn a few days previously) approximately equal in volume to that taken for samples. In control studies saline alone was given.

Results

Control studies

In three subjects, the effect of two periods of tilting to 60° without administration of the converting enzyme inhibitor was studied to determine the reproducibility of the response.

Plasma renin activity at the end of 1 h at 60° was similar in the two tilts; the supine levels were, however, higher before the second tilt than they had been before the first (Table 1).

Plasma aldosterone was estimated in two of the subjects and rose during both periods of tilting, the increase during the two tilts being comparable.

Blood pressure changed by only small amounts, and the response was similar in the two tilts; heart rate rose on average from 52 to 71 beats/min in the first tilt and 53 to 68 beats/min in the second.

Effect of converting enzyme inhibitor

Four subjects were studied according to the standard protocol; one additional subject was pretreated with dexamethasone (2 mg on the night before; 1 mg before and 1 mg during the experiment) to exclude the possibility that ACTH made an important contribution to the changes in plasma aldosterone. When the level of converting enzyme blockade was assessed at the end of the experiment, 2-2.5 h after infusion of SQ 20,881, an average of four times the dose of angiotensin I was required to achieve a given pressor response.

Plasma renin activity. Changes in PRA were similar in the subject given dexamethasone to the other four, and the results have therefore been analysed together. In all five subjects, the administration of the inhibitor was followed by an increase in PRA at rest which rose from an average of 0.53 ng ml^{-1} h^{-1} to 1.48 ng ml^{-1} h^{-1} ($P < 0.031$) (Table 2). The level of PRA during tilting was also higher after the drug, the average level being 1.3 ng ml^{-1} h^{-1} at the end of the control tilt and 3.6 ng ml^{-1} h^{-1} at the end of the second tilt.

Aldosterone. Plasma aldosterone concentrations in the supine position showed no consistent change after administration of the inhibitor. In all subjects, including the one given dexamethasone, plasma aldosterone rose during tilting and the concentrations achieved at the end of the two tilts were not greatly different; there was thus no consistent inhibition of aldosterone secretion following the infusion of SQ 20,881.

Cortisol. Plasma cortisol concentration in the supine position showed no significant change after the drug; the level rose during both periods of tilting, but the response was not significantly different after the drug. In the subject given dexamethasone, plasma cortisol levels were suppressed throughout and did not respond to tilting.

Table 1 Control studies. The effect of two periods of tilting on the plasma renin activity (PRA), aldosterone and cortisol levels in three subjects

	PRA (ng angiotensin 1 ml^{-1} plasma h^{-1} at 37°C)										*Aldosterone (ng/100 ml)*						*Cortisol (μg/100 ml)*					
	Supine		*1st tilt*		*Supine*				*2nd tilt*		*Supine*		*1st tilt*	*Supine*		*2nd tilt*	*Supine*		*1st tilt*	*Supine*		*2nd tilt*
Subject	*30 min*	*60 min*	*30 min*	*60 min*	*60 min*	*90 min*	*120 min*	*150 min*	*30 min*	*60 min*	*30 min*	*60 min*	*60 min*	*90 min*	*150 min*	*60 min*	*30 min*	*60 min*	*60 min*	*90 min*	*150 min*	*60 min*
1	0.11	0.1	0.27	0.48	0.27	0.27	0.29	0.24	0.39	0.43												
2	0.23	0.17	0.52	0.89	0.45	0.49	0.45		0.72	0.77		9.4	25.7	5.4		19.9		16.5	18.1	13.2		10.9
3	0.31	0.26	1.32	3.38	0.83	0.73	0.68	0.60	2.05	3.65		15.0	26.5		9.6	21.0	9.4		16.3	18.5	7.0	18.5
mean	0.22	0.18	0.7	1.58	0.52	0.50	0.47	0.42	1.05	1.62												

Table 2 The effect of two periods of tilting on the plasma renin activity (PRA), aldosterone and cortisol levels in five subjects before and after the administration of SQ 20, 881, an inhibitor of angiotensin converting enzyme

	PRA (ng angiotensin 1 ml^{-1} plasma h^{-1} at 37°C)										*Aldosterone (ng/100 ml)*						*Cortisol (μg/100 ml)*					
	Before SQ 20,881					*After SQ 20,881*					*Before SQ 20,881*				*After SQ 20,881*		*Before SQ 20,881*				*After SQ 20,881*	
	Supine		*1st tilt*			*Supine*			*2nd tilt*		*Supine*		*1st tilt*	*Supine*		*2nd tilit*	*Supine*		*1st tilt*	*Supine*		*2nd tilt*
Subject	*30 min*	*60 min*	*30 min*	*60 min*	*60 min*	*90 min*	*120 min*	*150 min*	*30 min*	*60 min*	*30 min*	*60 min*	*60 min*	*90 min*	*150 min*	*60 min*	*30 min*	*60 min*	*60 min*	*90 min*	*150 min*	*60 min*
1	0.72	0.85	1.7	2.32	1.11	1.03	2.75	2.09	4.0	5.0	3.6	6.1	16.5	3.9	8.1	17.2	6.7	10.8	19.5	7.2	8.2	12.3
2*	0.28	0.27	0.67	0.67	0.56	0.89	2.35	2.55	3.52	2.59	2.0		13.7	4.3	2.7	26.8		1.4	1.4	1.4	1.8	2.3
3	0.28	0.32	1.75	2.4		0.32	1.65	1.9	6.75	7.84	6.3	10.4	55.3	19.6	20.0	34.5	5.9	6.3	26.7	15.5	5.9	21.9
4	0.05	0.07	0.17	0.25	0.07	0.08	0.07	0.12	0.2	0.72	2.3		8.1	6.6	7.7	12.0	7.1	7.6	21.4	16.2	13.8	21.0
5	0.23	0.27	0.45	0.9	0.4	0.33	0.65	0.75	1.06	1.71	14.7	18.3	36.4	38.3	17.1	22.6	5.9	5.9	26.7	16.7	8.6	28.3
	a	a		b	c		d	d		e	*	*	*	*	*	*	*	*	*	*	*	*
mean	0.31	0.33	0.95	1.3	0.53	0.53	1.49	1.48	3.1	3.6	5.8	11.6	26.0	14.5	11.1	22.0	6.4	7.6	23.6	13.9	9.1	20.8

* Mean excludes subject 2 who had been treated with dexamethasone.
The following differences are significant by the sign test (Siegel, 1956), b to average a, c to average d, e to b and e to average d ($P < 0.031$).

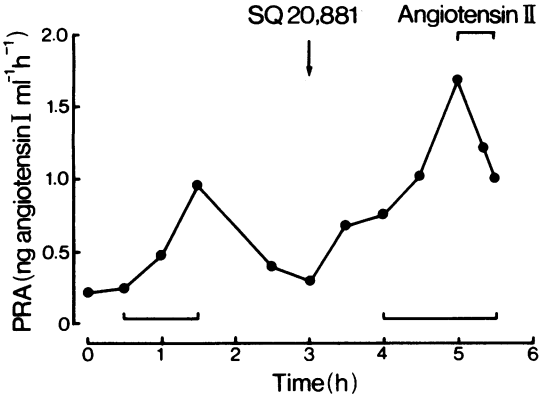

Fig. 1 Response of plasma renin activity (PRA) to tilting before and after administration of converting enzyme inhibitor (SQ 20,881, 0.5 mg/kg) in one subject. Infusion of angiotensin II (100 ng/min) during the last 30 min of the second tilt results in a rapid fall of the elevated PRA. The periods of tilting are shown by the lower brackets.

Heart rate. This rose from an average of 57 beats/min to 75 beats/min during the first tilt and 64 beats/min to 79 beats/min in the second.

Arterial pressure. Systolic pressure tended to fall during the tilt, the changes were inconsistent; diastolic pressure showed on average little change. The response during the second tilt was similar to that during the first.

Effect on plasma renin activity of infusing angiotensin I and II

In order to clarify the effect of changes in plasma angiotensin II concentration on PRA, one subject was maintained in the upright position after completion of the second tilt and angiotensin II was infused intravenously at 100 ng/min for 30 min; PRA fell progressively over the time of the infusion (Figure 1). In another study in which angiotensin I was infused in a supine subject at a rate of 800 ng/min for 60 min, plasma renin activity fell from a control of 0.19 ng ml^{-1} h^{-1} to 0.07 ng ml^{-1} h^{-1}; when the infusion was stopped, plasma renin activity rose to 0.36 ng ml^{-1} $hour^{-1}$.

Discussion

The increase in plasma renin activity and aldosterone observed in control studies after tilting to 60° is similar to that previously reported by others (Gordon *et al.*, 1966); an increase in plasma cortisol with tilting has also been observed before (Balikian, Brodie, Dale, Melby & Tait, 1968; Mitra, Genuth, Berman & Vertes, 1972). The control studies in which the subject was tilted on two occasions without any intervention showed that the response of both PRA and aldosterone was reproducible. The blockade of conversion of angiotensin I to angiotensin II achieved by the converting enzyme inhibitor was comparable to that found in previous studies in which a dose of 0.5 mg/kg was found to reduce the pressor effect of angiotensin I to approximately 10% of control immediately after injection, rising to about 20% after 3 h (Collier *et al.*, 1973).

The most important finding of the present study is that administration of converting enzyme inhibitor leads to a rise of PRA above the respective control levels in both supine and upright postures. Elevation in resting PRA has previously been noted after administration of the inhibitor in rats (Bing, 1973) and the rise in PRA induced by renal artery constriction in the dog is also augmented (Miller, Samuels, Haber & Barger, 1972). The possibility that the rise in PRA in the second half of the experiment reflected a fall in blood volume as a result of repeated blood sampling was excluded by the experiments in which all blood withdrawn was replaced by at least an equal volume of the subject's own blood. It appears likely that the rise in PRA was secondary to a fall in plasma angiotensin II concentration (changes in angiotensin II were not measured but plasma levels would be expected to fall when converting enzyme is inhibited). This possibility was supported by the observation that infusion of angiotensin II led to a rapid fall in the elevated PRA despite the fact that the subject was maintained in the upright position. Furthermore, PRA fell in the unblocked subject infused with angiotensin I at rest, but subsequently rose above control levels when the infusion was discontinued.

The absence of any demonstrable change in the response of aldosterone to tilting after administration of converting enzyme inhibitor is surprising if, as has been suggested, the increased secretion is mediated solely by angiotensin II. It is possible that the rise in PRA which occurred after the drug offset to a large extent the expected fall in angiotensin II, and this could account for the lack of significant changes in the aldosterone response.

It is also possible, however, that the acute changes in the distribution of blood volume induced by the upright posture is responsible for the plasma aldosterone response by a mechanism quite separate from any increase in renin secretion. Mitra *et al.* (1972) studied anephric patients and found that despite their inability to increase PRA

there was a significant rise in aldosterone in response to posture. These authors also noted an increase in cortisol in response to posture, but this was not consistent in all patients and did not correlate with the increase in aldosterone levels so that ACTH release could not be regarded as the only alternative stimulus for aldosterone. The possibility that the changes in aldosterone in our experiments were mediated predominantly by increased secretion of ACTH is made unlikely by the findings that prior administration of dexamethasone suppressed the cortisol response but had no effect on aldosterone.

A fall in PRA in response to infusion of angiotensin II has previously been noted in man (de Champlain, Genest, Veyratt & Boucher, 1966). The present study complements these findings by showing that PRA rises when converting enzyme is inhibited and generation of angiotensin II is reduced. Furthermore, it has recently been shown that blockade of angiotensin II receptors also leads to a rise in PRA (Brunner, Gavras, Laragh & Keenan, 1973). It thus appears that negative feedback from angiotensin II provides a sensitive mechanism for the control of PRA.

The rise in PRA activity after converting enzyme inhibition or administration of an angiotensin II receptor blocker has possible clinical implications. If, as has been suggested (Brunner, Laragh, Baer, Newton, Goodwin, Krakoff, Bard & Bühler, 1972), raised levels of renin were to have a direct effect in increasing the risk of certain complications of hypertension, the use of drugs of this type would be undesirable. The role of renin as a cause of cardiovascular complications has, however, been disputed (Doyle, Jerums, Johnston & Louis, 1973).

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