Adrenal angiotensin II receptors and vascular reactivity to angiotensin II in the rat during continuous inhibition of angiotensin converting enzyme

J. A. MILLAR,* D. J. CASEY and C. I. JOHNSTON
Monash University Department of Medicine, Prince Henry's Hospital, Melbourne, Victoria, Australia

Summary
1. The effect of continuous infusion of captopril (80 μg/h) for up to 5 days on blood pressure, adrenal angiotensin II receptors and vascular reactivity to exogenous angiotensin II, arginine vasopressin and noradrenaline was studied in the rat.

2. In treated rats, blood pressure decreased transiently to a minimum after 2 days (~18 mmHg). Vascular reactivity to angiotensin II, but not to arginine vasopressin, was also increased significantly after 2 days, but vascular reactivity to noradrenaline decreased. After 5 days, vascular reactivity to angiotensin II had returned to normal and was similar to that of sham-treated controls. Adrenal angiotensin II receptor concentrations decreased significantly after 1 and 2 days, but at 5 days were again similar to controls. There was no change in receptor affinity.

3. Converting enzyme inhibition with captopril causes transient specific changes in adrenal angiotensin II receptors and vascular reactivity. The receptor and vascular effects may facilitate and oppose, respectively, the early changes in blood pressure with captopril, but a long-term contribution from either is unlikely.

Key words: aldosterone, angiotensin II receptors, blood pressure, captopril, converting enzyme inhibition, vascular reactivity.

Introduction

The development of a specific inhibitor of angiotensin converting enzyme which is effective when taken orally (captopril; D-3-mercapto-2-methylpropanoyl-L-proline; SQ 14 225) has been a major advance in therapeutics (Gavras, Brunner, Turini, Kershaw, Tiff, Cuttelod, Gavras, Vukovich & McKinstry, 1978; Johnston, Millar, McGrath & Matthews, 1979). The mechanism of action of this drug appears to be more complex than the early assumption that its effect on blood pressure was due solely to decreased generation of angiotensin II (ANG 11) (Ondetti, Rubin & Cushman, 1977) and it is likely that specific local actions arising from inhibition of converting enzyme in tissues are important determinants of the overall physiological effect (Clappison, Millar, Casley, Anderson & Johnston, 1980; Johnston, Clappison, McGrath, Matthews, Millar & Anderson, 1980). ANG II has a trophic action on the aldosterone-producing cells of the adrenal zona glomerulosa (Laragh, Angers, Kelly & Lieberman, 1960) in addition to having a direct vasopressor effect. ANG II receptors in the adrenal cortex have been defined by radioligand binding (Goodfriend & Lim, 1970) and are sensitive to prevailing levels of ANG II, such that receptors are 'up-regulated' by raised circulating levels of the agonist (Aguilera, Hauger & Catt, 1978; Hauger, Aguilera & Catt, 1978; Devynck, Pernollet, Matthews, MacDonald, Raisman & Meyer, 1979). Inhibition of ANG II formation by captopril may thus cause a decrease in adrenal ANG II receptors. Such an effect might contribute to the fall in aldosterone levels seen with captopril (McCaa, Hall & McCaa, 1978) and have long-term importance in maintaining the therapeutic effect in hypertensive patients.

Vascular responsiveness to exogenous ANG II is also determined by prevailing levels of circulating ANG II, probably via changes in fractional receptor occupancy (Thurston & Laragh, 1975), and is also likely to change during treatment with captopril.

In this paper we report the effect of con-
tinuous administration of captopril for up to 5 days on adrenal ANG II receptors and vascular reactivity in the rat.

Materials and methods

Experimental protocol

Female Sprague-Dawley rats (200 ± 20 g) were used. Captopril was infused continuously (80 μg/h) by means of an osmotic mini-pump (Alza, Palo Alto, California, U.S.A.) implanted in the abdominal cavity under ether anaesthesia. Sodium chloride solution (0.15 mol/l) was used as the vehicle. Sham-operated rats studied simultaneously served as controls. Blood pressure was measured at daily intervals by tail plethysmography.

ANG II receptor studies

After 1, 2 and 5 days of captopril infusion, both captopril-treated and sham-operated control rats were anaesthetized with ether. Blood (5 ml) was sampled from the inferior vena cava and bilateral adrenalectomy was performed. Specific ANG II binding to an adrenal membrane preparation was measured at 26°C by the method of Pernollet, Devynck, Matthews & Meyer (1977), except that 125I-labelled ANG II of high specific radioactivity (800 μCi/μmol) was used instead of 3H-labelled ANG II. Tubes for measurement of total binding contained adrenal membranes (50 μl), 125I-labelled ANG II at concentrations from 5 × 10⁻⁹ mol/l to 10⁻¹⁰ mol/l (200 μl), and 0·1 mol of histidine-HCl/l, pH 7·1 (200 μl). Non-specific binding was measured in tubes which contained 0·1 mol of ANG II/l. Specific binding was defined as the difference between total and non-specific binding. Receptor concentration and affinity were determined by Scatchard analysis of the binding data (Scatchard, 1949) or as specific binding (fmol/mg of protein) at a ligand concentration of 2 × 10⁻⁹ mol/l.

Vascular reactivity to ANG II

Rats treated with captopril as above were anaesthetized with pentobarbitone and prepared for ANG II bioassay by the method of Peart (1955). ANG II was administered intravenously in incremental doses from 1 to 25 pmol, each as a bolus, and the pressor dose–response curves were constructed. The specificity of any change observed was tested by constructing similar curves for arginine vasopressin at similar doses and also for noradrenaline at doses of 150–600 pmol (50–200 ng).

Laboratory methods

Plasma renin concentration was measured by the method of Poulsen & Jorgensen (1974). ANG I and II in rat blood were measured by specific radioimmunoassays by the methods of Johnston, Mendelsohn & Casley (1969) and Boyd, Landon & Peart (1969) respectively, after extraction of both peptides into ethanol as described by Mashford & Roberts (1972).

Protein was assayed by the method of Lowry, Rosebrough, Farr & Randall (1951).

Statistics

Differences in receptor concentration between sham-operated and captopril-treated animals were tested for statistical significance by the unpaired t-test. The null hypothesis was rejected when P < 0·05.

Results

Effect of continuous administration of captopril on blood pressure

There was a significant decrease (P < 0·01) in blood pressure in treated rats, with a minimum at 2 days, from 129 ± 4 to 111 ± 1 mmHg. After 5 days, blood pressure in the treated group had returned to the level in control rats.

Criteria of converting enzyme inhibition

The degree of converting enzyme inhibition was assessed by reference to circulating levels of ANG I and ANG II, plasma renin concentration and the pressor response to exogenous ANG I. Values for plasma renin concentration, ANG I and ANG II in captopril and sham rats at 2 days were 83 ± 7 and 31 ± 5 ng of ANG I h⁻¹ ml⁻¹ of plasma for plasma renin concentration, 279 ± 36 and 88 ± 24 pg of ANG I/ml of blood and 30 ± 3 and 47 ± 11 pg of ANG II/ml of blood respectively. Corresponding values at 5 days were 53 ± 20 and 18 ± 4 ng h⁻¹ ml⁻¹, 180 ± 20 and 100 ± 47 pg of ANG I/ml and 41 ± 4 and 72 ± 17 pg of ANG II/ml. In addition, the pressor effect of exogenous ANG I was decreased in captopril-treated rats. The ratios of doses required to increase blood pressure by 10 mmHg in treated and control rats at 2 and 5 days were 6·6 and 4·6 respectively.
Vascular and adrenal effects of captopril

Effect of captopril on adrenal ANG II receptors

Receptor affinity in captopril-treated and sham rats were similar at all three time intervals. There was a significant decrease in receptor concentration at 1 and 2 days in treated rats, with the maximum reduction (18%) occurring after 2 days (Fig. 1). Receptor binding at 5 days was 11% less in rats given captopril, but the difference was not statistically significant (0.1 < \( P < 0.05 \)). Receptor concentrations after 1, 2 and 5 days of captopril, measured at a ligand concentration of 2 \( \times 10^{-9} \) mol/l, were 162 ± 13, 131 ± 7 and 180 ± 11 fmol/mg of protein respectively, compared with corresponding values in sham-treated rats of 191 ± 16, 160 ± 9 and 203 ± 14 fmol/mg.

Effect of captopril on the vascular reactivity to ANG II

Vascular reactivity was determined after 2 and 5 days of captopril. After 2 days there was an increase in the vascular sensitivity to ANG II in treated rats, with a significant parallel leftward shift in the pressor dose–response curve and a corresponding ED\(_{50}\) ratio (untreated/treated) of 5.0. However, after 5 days of captopril infusion the pressor response to ANG II was similar in both groups. The pressor dose–response curves for arginine vasopressin did not change during captopril administration. The pressor responses to 150, 300 and 600 pmol of noradrenaline at 2 days in treated rats was 11 ± 1, 18 ± 1 and 32 ± 2 mmHg (\( n = 4 \)). Corresponding values in sham rats were 15 ± 2, 30 ± 4 and 45 ± 4 mmHg respectively. Thus the vascular reactivity to noradrenaline was decreased in rats given captopril.

Discussion

The purpose of this study was to identify specific effects of captopril on the adrenal gland and vascular bed which may modulate the hypotensive effect of converting enzyme inhibition. The similarities in the temporal changes in blood pressure, adrenal ANG II receptors and vascular reactivity were striking. However, it is possible that the decrease in blood pressure we observed in captopril-treated rats was related only to a temporary dependency of blood pressure on ANG II induced by laparotomy. Administration of captopril in this circumstance might be expected to cause a fall in pressure.

Several studies have demonstrated increased adrenal ANG II receptor concentration in response to raised circulating levels of ANG II produced either by dietary salt depletion for up to 4 days (Aguilera et al., 1978) or up to 6 weeks (Douglas & Catt, 1976; Devynck et al., 1979) or by infusion of synthetic peptide (Hauger et al., 1978). Hence it is likely that the adrenal ANG II receptor is positively regulated by its agonist. However, the effect of decreased ANG II levels on the receptors has been less extensively studied. Devynck, Pernollet, MacDonald, Matthews, Raisman & Meyer (1978) found that adrenal angiotensin receptors in rats were unchanged after 4 weeks of a high-salt diet. Earlier periods were not studied, and it is possible that transient changes did occur. Converting enzyme inhibitors such as captopril offer a novel means of testing the effect of decreased ANG II levels on adrenal angiotensin receptors, independently of the state of salt balance. This study has shown that adrenal angiotensin receptors are indeed sensitive to decreased agonist concentrations and respond by a transient decrease in receptor concentration without change in affinity. Since changes in receptor concentrations of this magnitude are known to be associated with decreased adrenal responsiveness to ANG II with respect to aldosterone secretion (Aguilera et al., 1978), this effect is likely to contribute to the early fall in blood pressure seen with captopril. In similar experiments, Catt, Aguilera, Capponi, Fukita, Schirar & Fakunding (1979) have shown that the increase in adrenal angiotensin receptors, which
occurs with dietary salt depletion, is prevented by concurrent administration of captopril, a finding which is consistent with the present study.

ANG II increases blood pressure when infused continuously at doses which are sub-pressor when given acutely (Reigger, Slack, Casals-Stenzel, Brown, Fraser, Lever, Millar, Morton, Robertson & Tree, 1978). The mechanism of this property is not known, but possibly results from 'up-regulation' of angiotensin receptors with consequent increased mineralocorticoid release, or increased vascular responsiveness to ANG II. Conversely, long-term administration of captopril, with chronically depressed levels of ANG II, may produce a decrease in blood pressure due to a reversal of this effect. It was therefore of interest that receptor concentrations in captopril-treated rats were still decreased after 5 days. Although this was not statistically significant, further studies at this and longer periods of captopril administration are required. In the present studies, we did not observe any change in receptor affinity. Available evidence suggests that changes in affinity do occur early in the course of sodium restriction (Aguilera et al., 1978) but have not been documented with salt loading.

Our results for vascular reactivity are more difficult to interpret. The response of the vascular tree to any pressor agent depends on many factors, including ambient blood pressure, prevailing levels of the hormone under study, degree of receptor occupancy and facilitation by other hormones. In the present study, vascular sensitivity was increased in captopril-treated rats after 2 days of treatment. It is likely that this was a specific effect due to decreased levels of angiotensins, since the responsiveness to arginine vasopressin, a peptide of similar pressor potency to ANG II, was not similarly increased. Indeed, vascular sensitivity to noradrenaline at 2 days actually decreased. This interesting finding was first noted by Okuno, Kondo, Konishi, Saruta & Kato (1979), who suggested that captopril has a direct relaxing effect on vascular smooth muscle.

It is tempting to suggest that the increased vascular sensitivity was due to changes in vascular receptors which were parallel to those seen in the adrenal. Unfortunately it is not possible to test this hypothesis directly by radioligand binding. Angiotensin receptors in rabbit aorta and rat uterine smooth muscle, used previously as a model for arteriolar receptors, exhibit responses to changes in salt balance which are contrary to those occurring in adrenal receptors (Devynck et al., 1978, 1979) suggesting that receptor changes may not mediate the increased vascular sensitivity. An increase in the pressor dose–response relationship for ANG II occurs during salt loading when ANG II levels are suppressed and the converse obtains in salt depletion. These effects have been explained by changes in the fractional occupancy of receptors (Thurston & Laragh, 1975) and such a situation may have obtained in the present study. Whatever the mechanism, the presence of increased vascular sensitivity during the initial stages of continuous captopril administration tends to oppose any fall in blood pressure and may be regarded as a protective mechanism.

In summary, adrenal ANG II receptor concentrations and vascular sensitivity to exogenous angiotensin increased transiently during continuous administration of captopril in the rat. The receptor and vascular changes may facilitate and oppose, respectively, the early effects of captopril on blood pressure. These studies emphasize that the mode of action of captopril is complex and involves tissue effects not directly related to converting enzyme inhibition.

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