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Effect of captopril on changes in plasma noradrenaline induced by sodium nitroprusside

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1 There is much animal data to suggest that angiotensin II has a regulatory role in noradrenaline release. We sought evidence for such a mechanism in man by pretreating six normal volunteers with captopril (50 mg) or placebo and then infusing them with incremental doses of sodium nitroprusside.

2 Pretreatment with captopril had no significant effect on the mean arterial pressure, heart rate or plasma noradrenaline response to sodium nitroprusside, despite increasing plasma renin activity.

3 This suggests that in normotensive salt replete man, normal levels of angiotensin II do not exert any tonic effect on noradrenaline release.

Keywords converting enzyme inhibitors angiotensin II sympathetic nervous system nitroprusside sodium

Introduction

Many investigators have examined the independent roles of the sympathetic nervous system and the renin-angiotensin system in the control of blood pressure. Recently, however, evidence has accumulated suggesting that there is a significant interaction between these two control mechanisms (Zimmerman *et al.*, 1984). This evidence comes mainly from studies in isolated organs and in intact animals where exogenous angiotensin II has been shown to facilitate noradrenaline release (Zimmerman, 1981; Zimmerman *et al.*, 1972). In addition, in pithed rats angiotensin converting enzyme inhibitors have been shown consistently to attenuate the pressor response to sympathetic activation, although whether this is a prejunctional or postjunctional effect remains uncertain (Antonaccio & Kerwin, 1980; Hatton & Cough, 1982). There is, however, little data on whether such a mechanism also occurs in man.

A closely related and much investigated question is why angiotensin converting enzyme inhibitors appear able to produce an antihypertensive effect without causing a reflex tachycardia and reflex sympathoadrenal activation (Millar *et al.*, 1982). This surprising property of ACE inhibitors has arisen by comparing them with vasodilator drugs such as nifedipine, hydralazine and nitroprusside (Murphy *et al.*, 1982). One possibility which arises is that the reduced angiotensin II caused by ACE inhibition causes inhibition of noradrenaline release.

To investigate whether angiotensin II does control noradrenaline release in man, we have studied the effect of captopril on the profound reflex sympathoadrenal activation which accompanies the infusion of sodium nitroprusside in man.

Methods

The subjects were six normotensive males ranging in age from 26 to 34 years and in body weight

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Table 1 Systolic and diastolic blood pressure, heart rate and plasma noradrenaline before and 1 h after captopril (50 mg orally) or placebo. Results are expressed as mean ± s.e. mean (n = 6).

	SBP (mm Hg)		*DBP (mm Hg)*		*HR (beats min^{-1})*		*NA (ng l^{-1})*	
	Placebo	*Captopril*	*Placebo*	*Captopril*	*Placebo*	*Captopril*	*Placebo*	*Captopril*
Before	111 ± 4	112 ± 2	63 ± 2	64 ± 2	65 ± 3	64 ± 3	434 ± 16	425 ± 33
After	113 ± 4	112 ± 2	67 ± 2	63 ± 3	66 ± 2	66 ± 3	419 ± 25	419 ± 31

SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, NA = noradrenaline. None of the above data are significantly different between placebo and captopril.

from 56 to 85 kg. Each subject gave written informed consent to the procedure after it had been approved by the Research Ethics Committee of the Royal Postgraduate Medical School.

The six subjects took no cardiovascular drugs for at least 14 days and no other drugs for at least 3 days prior to each of the two study days which were themselves at least 1 week apart. Although sodium intake was not strictly controlled, subjects were instructed to maintain the same approximate sodium intake for 5 days prior to each study day and this was checked by measuring baseline values of plasma renin activity on each study day. They attended at 13.30 h after a light breakfast but had omitted luncheon.

The study was carried out using 50 mg of encapsuled captopril or matching placebo in a randomised double-blind crossover fashion. Intravenous cannulae were inserted into the antecubital veins of both arms, one for infusion and the other for blood sampling. After 15 min supine rest two sets of basal measurements of blood pressure and heart rate and two blood samplings were made at 5 min intervals. The mean results were taken as the control values.

The subjects then took a capsule (captopril 50 mg or matching placebo) with 30 ml of water and remained in bed supine for a further 60 min. After two further sets of baseline readings, the subjects received an incremental infusion of sodium nitroprusside starting with 0.2 μg kg^{-1} min^{-1} and increasing to 0.5, 0.8, 1.2, 3.0 and 5.2 μg kg^{-1} min^{-1} at 5 min intervals to a maximum permissible heart rate of 100 beats min^{-1} or a maximum fall in blood pressure of 25 mm Hg.

Blood pressure and heart rate were recorded and blood samples for catecholamine determinations were drawn into heparinized tubes at each dose. Final readings were made after 20 min recovery. Blood samples for estimation of plasma renin activity were taken into EDTA-containing bottles at the basal readings, at the top dose and on recovery.

Recumbent blood pressure was measured in duplicate using a semi-automatic sphygmomanometer (Roche Arteriosonde). The ECG was monitored continuously throughout the investigation and the heart rate was read from the ECG printout.

Blood samples for noradrenaline, adrenaline and renin were kept on ice until centrifugation at 4°C. Plasma was stored at −80°C and catecholamines were assayed by a double-isotope enzymatic technique (Brown & Jenner, 1981) and plasma renin by radioimmunoassay.

Nitroprusside solutions, 100 mg ml^{-1} were prepared immediately before use from Nipride ampoules at 5 g l^{-1} dextrose solution.

The dose-response curves to sodium nitroprusside were analysed by a three factorial analysis of variance with the three factors being placebo or captopril, the dose of nitroprusside and the identity of each subject. In addition, paired t-tests were performed at each dose to detect any significant differences at matched time points.

Results

Table 1 shows the baseline readings of systolic and diastolic blood pressure, heart rate and noradrenaline before and 1 h after 50 mg oral captopril or placebo. There were no changes in any of these observations in response to captopril.

The maximum responses to sodium nitroprusside are shown in Figure 1. As their highest nitroprusside dose, three subjects tolerated 5.2 μg kg^{-1} min^{-1} on both occasions, two 3.0 μg kg^{-1} min^{-1} and one 1.8 μg kg^{-1} min^{-1}. The latter subject had a baseline heart rate of 72–78 beats min^{-1}. The maximum induced changes under captopril were not different from the changes under placebo nor were there differences in the infusion rate. No significant differences between captopril or placebo were seen in systolic blood pressure, mean arterial pressure, diastolic blood pressure, heart rate or plasma noradrenaline (both by ANOVA and paired t-tests).

Figure 2 shows the plasma renin activity (PRA). The similar starting values on the two study days suggest that there were no major

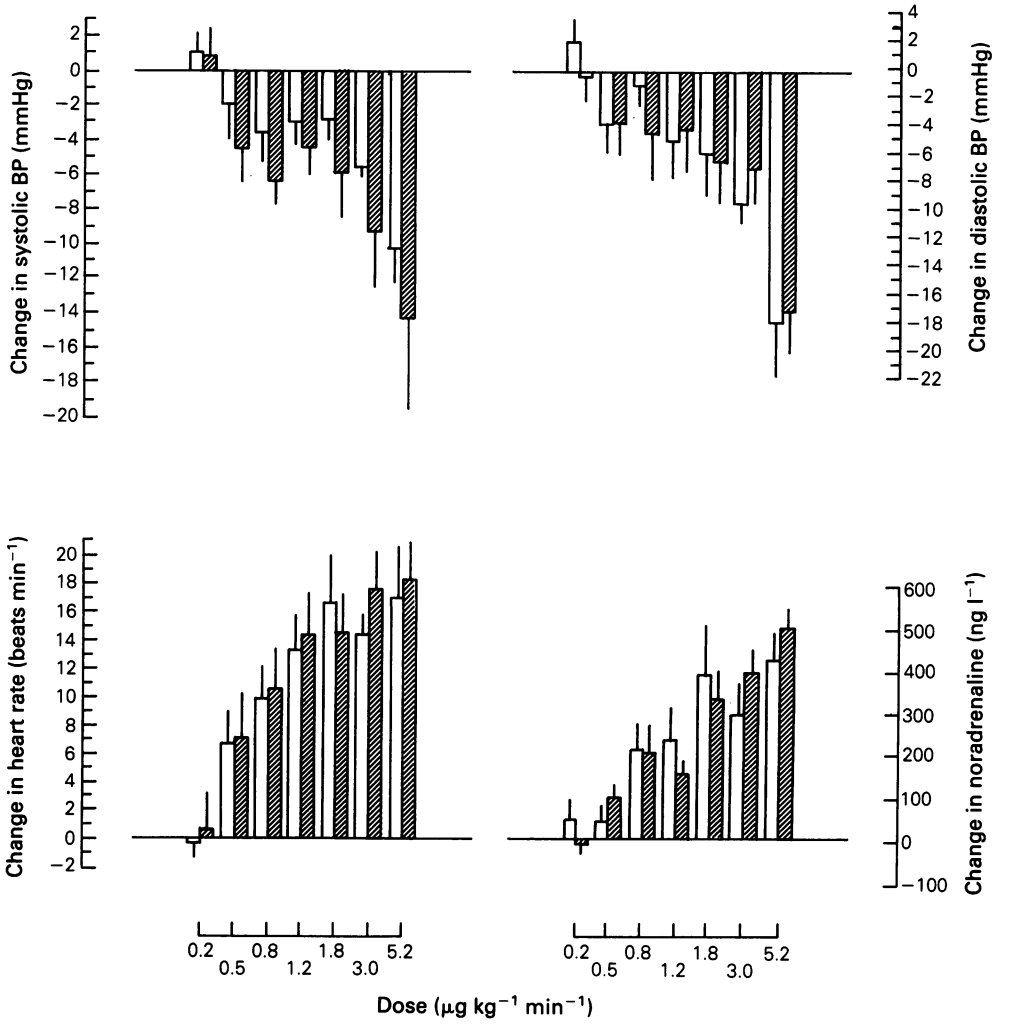

Figure 1 Haemodynamic and plasma noradrenaline responses (mean ± s.e. mean) to nitroprusside-induced vasodilation with (▨) and without (□) converting enzyme inhibition by captopril.

differences in sodium intake. On the placebo study day PRA rose insignificantly from 0.85 ± 0.1 to 1.35 ± 0.3 ng ml^{-1} h^{-1} but returned to baseline on recovery. Captopril increased plasma renin activity from 0.9 ± 1.0 to 2.0 ± 0.5 ng ml^{-1} h^{-1} at rest but increased it to nearly ten-fold the baseline value during the nitroprusside infusion to 7.0 ± 1.5 ng ml^{-1} h^{-1}. PRA was still significantly elevated on recovery both compared to pre- and post-captopril baseline values. The initial captopril induced increase in plasma renin activity is evidence that converting enzyme inhibition had been achieved in this study.

Mean plasma potassium concentration was 3.6 ± 0.2 mmol l^{-1} and was not altered by captopril or by the nitroprusside infusion.

Discussion

Much investigation has been directed towards understanding the lack of reflex tachycardia with ACE inhibitors. Millar *et al.* (1982) showed clearly that in normotensive man, enalapril has no effect on plasma noradrenaline or on its increment due to bicycle exercise, Valsalva manoeuvre or cold pressor test. Each of these

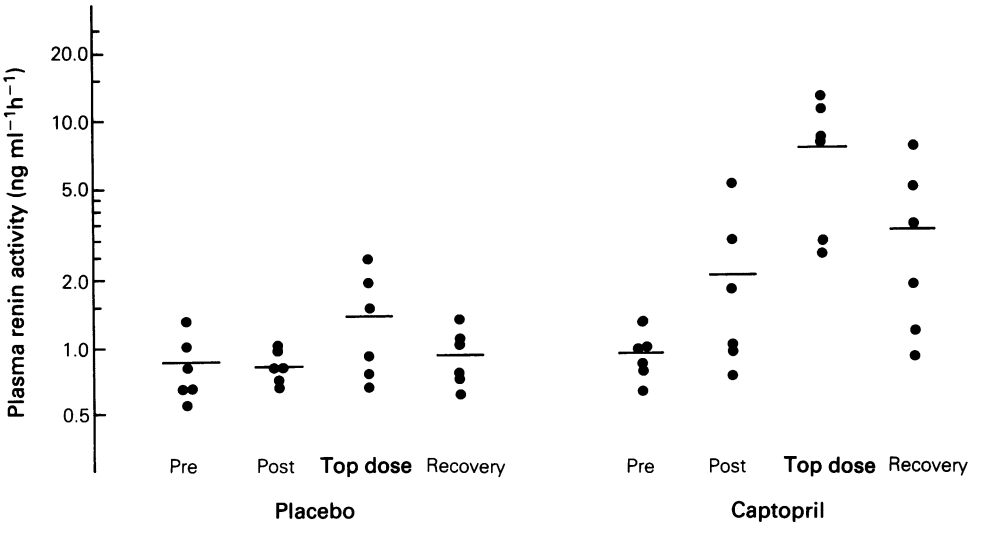

Figure 2 Plasma renin activity during nitroprusside-induced vasodilation with and without converting enzyme inhibition by captopril.

autonomic tests examines a different reflex pathway. However, the protocol of most relevance in investigating the reflex tachycardia question is to examine as in this study the effect of ACE inhibition on the baroreflex response to a vasodepressor stimulus. Captopril cannot be used alone to study baroreflex activity because it causes only a small hypotensive effect in normotensive subjects. We therefore used sodium nitroprusside as a stimulus to the baroreflex as we have previously found this to cause a large, dose-related increase in plasma noradrenaline (Dean *et al.*, 1980). We anticipated that if captopril had a prejunctional inhibitory effect, then the plasma noradrenaline increment with nitroprusside would be attenuated. Alternately, if as some data suggests (Imai *et al.*, 1982), captopril had a postjunctional inhibitory effect reducing responsiveness to noradrenaline, then excess noradrenaline might have been released to compensate, which would have been detected by a greater increment in plasma noradrenaline during nitroprusside. If both a prejunctional and a postjunctional effect were present and these two effects were of equal magnitude, it is conceivable that they might cancel each other out so that the nitroprusside induced increase in plasma noradrenaline was unaffected by captopril. This is in fact what we observed, although it is equally possible that captopril had neither a prejunctional nor a postjunctional effect.

Although we did not measure plasma angiotensin II in our study, Millar *et al.* (1981) have previously shown that 50 mg of captopril orally to salt replete normal volunteers produces a 50% fall in plasma angiotensin II, a secondary increase in plasma renin activity but no change in arterial blood pressure. These latter two findings agree with our own study and leave little doubt that the large dose (50 mg) of captopril which we employed would have reduced angiotensin II levels. One further complicating factor is that captopril inhibits bradykinin metabolism and it is possible that bradykinin itself has postsynaptic vascular effects (Inokuchi & Malik, 1984). This criticism can be levelled against most studies with ACE inhibitors but resolution of this problem must await improved methodology for the estimation of bradykinin itself.

Five possible reasons have been suggested for the lack of reflex tachycardia with ACE inhibitors. They were thought either to interfere with noradrenaline release or to alter baroreflex sensitivity. This study has demonstrated that neither of these hypotheses can be substantiated. It has recently been suggested that enalapril alters the baroreflex set point without altering its sensitivity (Giudicelli *et al.*, 1985). As a fourth possibility, Campbell *et al.* (1985) have shown that captopril enhances parasympathetic tone, which would offset any reflex tachycardia. The fifth possibility is that ACE inhibitors are relatively weak vasodilators and certainly cause less skin flushing than nifedipine, hydralazine or nitroprusside (Murphy *et al.*, 1982; Dean *et al.*, 1980). In fact in this study, a large dose of captopril itself had no significant effect on blood pressure, despite increasing plasma renin acti-

vity, and it is therefore hardly surprising that reflex tachycardia was not observed. Many investigators have assumed that ACE (and more recently renin) inhibitors are exceptional in not producing reflex tachycardia but the real exception among antihypertensive drugs may in fact be the vasodilator agents. Their ability to stimulate the baroreflex may be because they not only reduce mean arterial pressure but because they also widen pulse pressure. Even the lack of reflex tachycardia with β-adrenoceptor blocking drugs could be more anomalous than originally suspected since vagal withdrawal could mediate such an effect even in the presence of a β-adrenoceptor blocker.

The hypothesis that endogenous angiotensin II had a regulatory effect on noradrenaline release has been extensively studied in animals, with some conflicting results. Positive results for such a mechanism have been principally demonstrated in studies where exogenous angiotensin II had been administered and in studies of pithed rats where plasma renin is already elevated approximately 5–10 times over that in normal animals (De Jonge *et al.*, 1982). It has therefore proven easier to demonstrate this mechanism in situations where the renin angiotensin system is already activated. Our own study suggests that normal circulating levels of AII do not exert any tonic influence on noradrenaline release as far as can be detected with the experimental model of nitroprusside induced noradrenaline release in man. This observation does not necessarily mean that noradrenaline release would not be regulated by angiotensin II in clinical situations where the renin angiotensin system is activated. In fact, ACE inhibition does cause a decrease in plasma noradrenaline in congestive heart failure (Wenting *et al.*, 1983). The complicating factor here is that ACE inhibition itself improves the patient's overall clinical state and this may well be what reduces sympathetic overactivity in this group of patients.

References

Antonaccio, M. J. & Kerwin, L. W. (1980). Evidence for prejunctional inhibition of norepinephrine release by captopril in spontaneously hypertensive rats. *Eur. J. Pharmac.*, **68**, 209–212.

Brown, M. J. & Jenner, D. A. (1981). Novel double isotope technique for enzymatic assay of catecholamines, permitting high precision, sensitivity and plasma sample capacity. *Clin. Sci.*, **61**, 591–598.

Campbell, B. C., Sturani, A. & Reid, J. L. (1985). Evidence of parasympathetic activity of the angiotensin converting enzyme inhibitor, captopril, in normotensive man. *Clin. Sci.*, **68**, 49–56.

Dean, C. R., Maling T., Dargie, H. J., Reid, J. L. & Dollery, C. T. (1980). Effect of propranolol on plasma norepinephrine during sodium nitroprusside induced hypotension. *Clin. Pharmac. Ther.*, **22**, 156–164.

De Jonge, A., Knape, T. A., Van Meel, J. C. A., Kalkman, H. O., Wilffert, B. & Thoolen, M. J. M. L. (1982). Effect of converting enzyme inhibition and angiotensin receptor blockade on the vasoconstriction mediated by α_1 and α_2-adrenoceptor stimulation in pithed normotensive rats. *Naunyn Schmiedeberg's Arch. Pharmac.*, **321**, 309–313.

Giudicelli, J. F., Berdeaux, A., Edouard, A., Richer, A. & Jacolot, D. (1985). The effect of enalapril on baroreceptor mediated reflex function in normotensive subjects. *Br. J. clin. Pharmac.*, **20**, 211–218.

Hatton, R. & Cough, D. P. (1982). Captopril interferes with neurogenic vasoconstriction in the pithed rat by angiotensin-dependent mechanisms. *J. cardiovasc. Pharmac.*, **4**, 116–123.

Imai, Y., Abe, K., Seino, M., Haruyama, T., Tajima, I., Yoshinaga, K. & Sekino, H. (1982). Captopril attenuates pressor responses to norepinephrine and vasopressin through depletion of endogenous angiotensin II. *Am. J. Cardiol.*, **49**, 1537–1539.

Inokuchi, K. & Malik, K. U. (1984). Inhibition by bradykinin of renal adrenergic effects in anaesthetised rats. *Am. J. Physiol.*, **246**, F387–F394.

Millar, J. A., Derkx, F. H. M., McLean, K. & Reid, J. L. (1982). Pharmacodynamics of converting enzyme inhibition: the cardiovascular, endocrine and autonomic effects of MK 421 (enalapril) and MK 521. *Br. J. clin. Pharmac.*, **14**, 347–355.

Millar, J. A., McGrath, B. P., Mathews, P. G. & Johnston, C. I. (1981). Acute effects of captopril on blood pressure and circulating hormone levels in salt replete and depleted normal subjects and essential hypertensive patients. *Clin. Sci.*, **61**, 75–83.

Murphy, M. B., Scriven, A.J.I., Brown, M. J., Causon, R. & Dollery, C. T. (1982). The effects of nifedipine and hydralazine induced hypotension in sympathetic activity. *Eur. J. clin. Pharmac.*, **23**, 479–482.

Wenting, G. J., Man in't Veld, A. J., Waittiez, A. J. J., Boomsa, F., Laird-Meeter, K., Simoons, M., Hugenholtz, P. G. & Schalekamp, M. A. D. H. (1983). Effects of captopril in acute and chronic heart failure. *Br. Heart J.*, **49**, 65–76.

Zimmerman, B. G. (1981). Adrenergic facilitation by angiotensin: does it serve a physiological function? *Clin. Sci.*, **60**, 343–348.

Zimmerman, B. G., Gomer, S. K. & Liao, J. C. (1972). Action of angiotensin on vascular adrenergic nerve endings: facilitation of norepinephrine release. *Fed. Proc.*, **31**, 1344–1350.

Zimmerman, B. G., Sybertz, E. J. & Wang, P. C. (1984). Interaction between sympathetic and renin-angiotensin system. *J. Hypertension*, **2**, 581–587.

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