Br. J. Pharmac. (1982), **75**, 553–558

EFFECT OF CONVERTING ENZYME INHIBITION ON THE RENAL HAEMODYNAMIC RESPONSES TO NORADRENALINE INFUSION IN THE RAT

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1 The renal haemodynamic responses to a close arterial infusion of noradrenaline (29.7–177.9 nmol $kg^{-1} h^{-1}$) were measured in rats anaesthetized with pentobarbitone. Systemic blood pressure was unaffected by noradrenaline infusion at this dose level. Renal blood flow was significantly reduced by 16% while glomerular filtration rate remained unchanged. These responses resulted in a rise in filtration fraction of some 10%.

2 In a separate group of animals, noradrenaline infusion in this manner and at a similar dose rate increased plasma renin activity approximately 3 fold.

3 Continuous infusion of the angiotensin converting enzyme inhibitor, teprotide (3.36 μmol $kg^{-1} h^{-1}$), had no measurable effect on systemic blood pressure, renal blood flow, glomerular filtration rate or filtration fraction.

4 Infusion of noradrenaline into these animals receiving teprotide caused a significant fall in renal blood flow of 16%. There was a fall in glomerular filtration rate of some 17% which was significantly different from the response observed in the animals not receiving teprotide. There was a consequent small but insignificant fall in filtration fraction.

5 These data show that the regulation of glomerular filtration rate in response to the vasoconstrictor drug, noradrenaline, is partly mediated via the renin-angiotensin system. They provide evidence for a role of intrarenal angiotensin II in regulating glomerular filtration by causing efferent arteriolar vasoconstriction.

Introduction

Many early studies of renal function showed that infusion of adrenaline or noradrenaline (Brod, 1973) or reflex activation of the sympathetic nervous system (Renkin & Gilmore, 1973) caused a reduction in renal blood flow, a somewhat smaller fall in glomerular filtration rate with a consequent rise in filtration fraction. The mechanisms involved in causing these adrenergically mediated responses are not clear at present.

The kidney is extensively innervated by the sympathetic nervous system with nerve endings not only on the smooth musculature of the arterioles but also on the tubules themselves (Barajas, 1978). Functionally, activation of the renal nerves causes a reduction in renal blood flow, an α-adrenoceptor response (Coote, Johns, MacLeod & Singer, 1972), and the release of renin from the juxtaglomerular cells, a β-adrenoceptor response (Reid, Morris & Ganong, 1978).

Previous work from this laboratory, in which the cat was used, showed that part of the renal haemodynamic response to renal nerve stimulation is dependent on the intrarenal production of angiotensin II. Thus the ability to maintain glomerular filtration rate at control values during renal nerve stimulation was impaired following inhibition of the renin-angiotensin system (Johns, Lewis & Singer, 1976; Johns, 1979; 1980). Such an effect was consistent with the suggestion of locally generated angiotensin II having a preferential site of action at the efferent arteriole.

The possibility arises that the renal haemodynamic responses observed following administration of adrenaline or noradrenaline could be due not only to adrenergic vasoconstriction but also in part to the local release of renin and generation of angiotensin II. The present investigation was an attempt to study the role of the renin-angiotensin system under these conditions. Noradrenaline was administered to rats as a close arterial infusion and a comparison was made of the renal haemodynamic responses under normal conditions and following inhibition of converting enzyme to block angiotensin II production.

Methods

Male albino Sprague-Dawley rats, in the weight

0007-1188/82/030553-06 $01.00

range 300–350 g, were used. Anaesthesia was induced with sodium pentobarbitone, 240 μmol/kg intraperitoneally. A carotid artery was cannulated for blood pressure measurement (Bell and Howell transducer linked to a Devices M2 recorder) and removal of blood samples. Cannulation of the jugular vein allowed infusion of saline (150 mmol/l sodium chloride) at 6 ml/h.

The left kidney was exposed by means of a ventral mid-line incision. Renal venous blood was collected from a cannula placed in the left adrenal vein. Urine was collected by means of a cannula in the left ureter. A cannula was placed in the aorta, via the femoral artery, such that its tip lay 2–3 mm rostral to the left renal artery. A ligature was tied around the aorta just caudal to the left renal artery. This allowed uninterrupted blood flow to both kidneys but the major portion of any infused noradrenaline to go to the left kidney.

Renal function measurements

Following completion of surgery, 2 ml of a primer solution was given containing inulin, 10 mg/ml, and *p*-amino-hippuric acid (PAH), 26.8 mmol/l, in saline and the saline infusion changed to one containing 10 mg/ml inulin and 13.4 mmol/l PAH. Measurements were begun 1 h later. Arterial (0.35 ml) and renal venous (0.15 ml) blood samples were removed, centrifuged and the plasma stored, while the cells were resuspended in saline and infused back into the animal. Haematocrit was also determined.

The experimental procedure consisted of five 30 min clearance periods; two before, one during and two following noradrenaline infusion. At least 5 min were allowed from the beginning of the noradrenaline infusion via the femoral arterial cannula before the urine collection was begun for the subsequent clearance period. A similar period was allowed after the end of the noradrenaline infusion. A mean value of the two clearance periods before and the two periods after noradrenaline infusion was obtained and used as a control value which was compared to the single clearance value obtained during noradrenaline infusion. This was done in order to take into account any long term trends which might become apparent over the course of the experiment. Measurement of plasma levels of inulin and PAH were made at the beginning and end of each clearance period. Calculation of renal blood and plasma flow and glomerular filtration rate were as previously described (Johns *et al.*, 1976). Filtration fraction was determined from glomerular filtration rate and renal plasma flow.

Drug administration

Noradrenaline (Levophed, Winthrop) was given via the femoral arterial cannula in a dose ranging from 29.7 to 177.9 nmol $kg^{-1}h^{-1}$ (using an infusion rate of between 0.06 and 0.60 ml/h) which was sufficient to cause an approximate 16% reduction in renal blood flow. This dose level was obtained from preliminary tests and was taken as one quarter of that dose which caused a perceptible blanching of the kidney.

Converting enzyme inhibition was achieved by administration of teprotide (SQ 20881, E.R. Squibb & Sons Ltd) at 3.36 μmol $kg^{-1}h^{-1}$, intravenously, in the sustaining infusion of inulin and PAH. Infusion of teprotide was begun at the end of surgery and the effectiveness of inhibition was confirmed by demonstrating blockade of the blood pressure elevations to a bolus intravenous injection of 76.9 pmol angiotensin I (Schwartz Bioresearch) 15 min after the start of the teprotide infusion and again at the end of the experiment.

Renin measurements

A further group of animals were subjected to the same surgical preparation as for the renal function studies except that saline, at 6 ml/h, was infused for the duration of the experiment. Two hours after completion of surgery the first arterial blood sample

Table 1 Renal haemodynamic responses in the rat in response to noradrenaline infusion

	Control	*Noradrenaline infusion*	P
Mean arterial blood pressure (mmHg)	120.8 ± 2.7	124.1 ± 3.5	> 0.3
Renal blood flow (ml min^{-1} 100g^{-1} body wt.)	2.52 ± 0.21	2.11 ± 0.19	< 0.01
Glomerular filtration rate, (ml min^{-1} 100 g^{-1} body wt.)	0.46 ± 0.03	0.43 ± 0.02	> 0.1
Filtration fraction (%)	36.8 ± 2.4	40.7 ± 2.5	< 0.001

P indicates the statistical significance using a paired *t* test. Mean values ± s.e.mean of $n = 8$.

(0.7 ml) was removed for estimation of plasma renin activity (PRA). The close intra-arterial infusion of noradrenaline was begun as in the renal function studies. Forty minutes later a second arterial blood sample was removed for PRA measurement.

Arterial blood samples were collected into cooled syringes which had been rinsed with disodium edetate (160 mmol/l). They were immediately centrifuged at 4°C and plasma removed and stored in the deep freeze until assayed. The red cells were resuspended in saline and returned to the animal as quickly as possible.

The plasma was incubated at 37°C for 1 h in the presence of enzyme inhibitors (2,3 dimercaptopropanol and 8-hydroxyquinoline) to generate angiotensin I (Haber, Koerner, Page, Kliman & Purnode, 1969) which was estimated by radioimmunoassay (C.I.S. (U.K.) Ltd). PRA was expressed as pmol angiotensin I generated per ml plasma and per hour (pmol $ml^{-1}h^{-1}$).

Statistics

The absolute and percentage changes quoted in the text represent a mean of individual changes recorded in each animal. Mean values ± s.e.mean are used. Statistical analysis was undertaken using the paired Student's *t* test within groups and the unpaired Student's *t* test between groups. Differences were considered to be statistically significant when $P < 0.05$.

Results

The effects of close arterial infusion of noradrenaline into the kidney are given in Table 1. Over the 30 min of infusion at this dose level of noradrenaline there was no change in mean arterial blood pressure ($P > 0.3$). However, the infusion of noradrenaline caused a significant reduction in renal blood flow ($P < 0.01$) of approximately 16%. Glomerular filtration rate fell by some 7% which did not reach statistical significance ($P > 0.1$). These haemodynamic changes resulted in a significant rise in filtration fraction ($P < 0.001$) of some 10% during the noradrenaline infusion.

In a separate group of animals subjected to the same surgical procedures, the effectiveness of the close arterial noradrenaline infusion in inducing renin release was determined. Basal values of PRA were 1.61 ± 0.17 pmol $ml^{-1}h^{-1}$ which increased significantly ($P < 0.001$) after the 30 min of noradrenaline infusion to 5.61 ± 0.80 pmol $ml^{-1}h^{-1}$.

To assess the degree of converting enzyme blockade, the diminution of the blood pressure rises caused by an injection of angiotensin I was measured. Administration of a bolus intravenous dose of 76.9 pmol angiotensin I caused a rise in blood pressure of between 15 and 25 mmHg. Following 15 min infusion of teprotide, the blood pressure response to a further dose of 76.9 pmol angiotensin I was completely abolished. A test dose of 76.9 pmol angiotensin I at the end of the experiment was also without effect on the blood pressure.

The effects of close arterial infusion of noradrenaline into animals treated with teprotide are presented in Table 2. The control values of blood pressure, renal blood flow, glomerular filtration rate and filtration fraction in these animals were not significantly different from those in which teprotide had not been infused (all values of $P > 0.4$). Mean arterial blood pressure did not change when the noradrenaline was infused, a response similar to that observed in animals not given teprotide. Renal blood flow was significantly reduced ($P < 0.01$) by approximately 16%, again a response almost identical to that recorded in non-teprotide infused animals. However, glomerular filtration rate was significantly reduced ($P < 0.01$) by approximately 17% which was a response much larger than that measured in the absence of teprotide ($P < 0.05$). Consequently, filtration fraction did not change during the noradrenaline infusion which was significantly different ($P < 0.001$) from the increase recorded when the teprotide was not administered (Table 1).

Table 2 Renal haemodynamic responses in the rat in response to noradrenaline infusion in the presence of teprotide

	Control	*Noradrenaline infusion*	P
Mean arterial blood pressure (mmHg)	125.5 ± 5.6	126.3 ± 5.3	> 0.5
Renal blood flow, (ml min^{-1} 100 g^{-1} body wt.)	2.28 ± 0.23	1.94 ± 0.20	< 0.001
Glomerular filtration rate, (ml min^{-1} 100 g^{-1} body wt.)	0.43 ± 0.02	0.36 ± 0.02	< 0.01
Filtration fraction (%)	40.2 ± 2.6	39.2 ± 2.6	> 0.05

P indicates the statistical significance using a paired *t* test. Mean values ± s.e.mean of $n = 7$.

Discussion

This investigation was undertaken to examine the effects of noradrenaline infusion on renal haemodynamics and to determine whether the renin-angiotensin system was involved in mediating any of the responses. The experimental approach used allowed noradrenaline to be infused into the kidney without causing major changes in systemic blood pressure. The position of the cannula just above the origin of the renal artery from the aorta helped to ensure that most of the noradrenaline was directed towards the kidney and also it has been shown that the kidney can deactivate over 50% of all catecholamines passing through its circulation (Reid, Schrier & Earley, 1972). The dose of noradrenaline infused would represent a very high blood level and one which would not be normally encountered, although it may be comparable to that present at the neuroeffector junction.

The reduction of renal blood flow in response to the infused noradrenaline probably represented an α-adrenergic effect as our previous studies in the cat have shown that the renal vasoconstrictor effect of renal nerve stimulation can be blocked by the α-adrenoceptor antagonist, phentolamine (Coote *et al.*, 1972). The major resistance bed in the kidney comprises the afferent arterioles (Renkin & Gilmore, 1973) and the noradrenaline-induced reduction in renal blood flow is likely to represent an action at this point. However, if only the afferent arterioles had constricted, pressure within the glomerulus would have fallen resulting in a fall in glomerular filtration rate. Our data showed that during noradrenaline infusion there was no fall in glomerular filtration rate which probably indicated a concomitant vasoconstriction of the efferent arterioles such that filtration pressure was maintained. The possibility exists that this efferent arteriolar vasoconstriction could have been caused either directly by the infused noradrenaline or indirectly by some other mechanism.

The results of this study clearly showed that infusion of noradrenaline close arterially could cause a large rise in circulating levels of renin, reflecting increased secretion from the kidney. It is generally accepted that adrenergically induced renin release from the juxtaglomerular cells of the kidney is mediated via β-adrenoceptors (Reid *et al.*, 1978) and it is likely that such a mechanism was responsible for the increased PRA observed following noradrenaline in the present study. Although a reduction in renal blood flow can act as a stimulus to renin release (Davis & Freeman, 1976), under conditions of adrenergic vasoconstriction almost all the renin release can be blocked by specific β-adrenoceptor antagonists (Johns & Singer, 1974; Johns, 1981) and therefore in the present study it is probable that the reduction in renal blood flow would not contribute to the observed increases in PRA.

Two pharmacological options are available with which to inhibit the activity of the renin-angiotensin system but each has its own limitations. Analogues of angiotensin II have been developed which block angiotensin II receptor sites (Turker, Page & Bumpus, 1974) although many of these compounds have agonist activity (Marshall, 1976). Angiotensin converting enzyme inhibitors, such as teprotide (SQ 20,881) and captopril (SQ 14,225) can be used, however, the converting enzyme is identical to kininase II which is responsible for the breakdown of bradykinin (Erdös, 1977). Consequently, responses obtained in the presence of such drugs can be interpreted as either a lack of production of the vasoconstrictor angiotensin II or a potentiator of the vasodilator, bradykinin. The relative importance of this potentiation of bradykinin action is unclear and the subject of current debate (McCaa, 1979; Mimran, Casellas & Dupont, 1980; Textor, Brunner & Gavras, 1981). In spite of this reservation in the use of converting enzyme inhibitors, we chose to use teprotide as a previous study had shown that the effectiveness of angiotensin II receptor blocking drugs at intrarenal sites was very variable (Johns, 1980).

The dose of teprotide used in the present study completely blocked the increases in blood pressure caused by moderate doses of angiotensin I and was similar to that used in acute studies by Thurston & Swales (1978) and by ourselves in the cat (Johns, 1979). Teprotide had no measurable effect on systemic blood pressure, renal blood flow or glomerular filtration rate. Although inhibition of converting enzyme activity has been shown to decrease blood pressure and increase renal blood flow the magnitude of such responses are dependent on dietary sodium intake as well as the duration of drug administration (Arendshorst & Finn, 1977). It is possible that in the present study these acute responses in sodium replete rats would have been compensated by other mechanisms during the period following the start of drug administration and before the beginning of the experimental period.

Infusion of noradrenaline into animals receiving teprotide resulted in a reduction in renal blood flow almost identical to that observed in the animals not given teprotide. In contrast there was a much larger fall in glomerular filtration rate following converting enzyme inhibition, of approximately 17% as against the insignificant 7% in the animals not receiving teprotide. These results show that the noradrenaline was able to cause afferent arteriolar vasoconstriction, as in the control animals, but that the appropriate efferent arteriolar vasoconstriction was much reduced such that there probably was a fall in pressure within the glomerulus with the result that filtration

rate could not be maintained at its control level. One possible way of interpreting this response is that part of the action of noradrenaline is mediated at the efferent arteriole via local generation of angiotensin II by which means glomerular filtration rate can be regulated.

There is now increasing evidence from other species and experiments using other physiological approaches that intrarenal generation of angiotensin II is involved in the regulation of glomerular filtration rate. Studies by Hall and co-workers using the anaesthetized dog and the experimental manoeuvre of reduction in renal perfusion pressure over the autoregulatory range and of ourselves, using direct electrical stimulation of the renal nerves in the cat, have shown that the ability to regulate glomerular filtration rate is reduced following suppression of the renin-angiotensin system with high salt and DOCA (Hall, Guyton & Cowley, 1977), or blockade of neural renin release with a β-adrenoceptor antagonist (Johns *et al.*, 1976), administration of the receptor blocking drug 1-Sar, 8-Ile, angiotensin II (Hall, Guyton, Jackson, Coleman, Lohmeirer & Trippodo, 1977; Johns, 1980) or following inhibition of converting enzyme (Hall, Coleman, Guyton, Balfe & Salgado, 1979; Johns, 1979). As yet, evidence of such a role for intrarenal angiotensin II in the rat is indirect. Studies by Krahé, Hofbauer & Gross (1970) and Frega, Davalos & Leaf (1980) showed that administration of renin substrate to isolated perfused kidneys of the rat resulted in an increase in filtration fraction which the authors interpreted as an action at the efferent arteriole of endogenously produced angiotensin II. Further, Ichikawa & Brenner (1980) using micropuncture studies, reported the dependence of efferent arteriolar tone on angiotensin II production during reductions in renal perfusion pressure. More direct evidence in the rat was provided by a study in this laboratory (Ball & Johns, 1982) which showed that the regulation of glomerular filtration rate during direct electrical stimulation of the renal nerves was depressed following suppression of the renin-angiotensin system by administration of a high salt diet and DOCA.

The results presented here examine the renal haemodynamic responses to close arterial infusion of noradrenaline and their modification following inhibition of angiotensin converting enzyme. They provide further evidence to show that in the rat, intrarenal generation of angiotensin II is important for the regulation of glomerular filtration rate by modifying the tone of the efferent arteriole.

Generous supplies of teprotide were made available by Dr S.J. Lucania (Squibb & Sons Ltd).

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(*Received October 8, 1981.*)