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MECHANISMS INVOLVED IN THE RENAL RESPONSES TO INTRAVENOUS AND RENAL ARTERY INFUSIONS OF NORADRENALINE IN CONSCIOUS DOGS

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SUMMARY

1. The renal haemodynamic and glomerular filtration rate (G.F.R.) responses to intravenous and intrarenal infusions of noradrenaline were studied in conscious dogs, either with or without prior blockade of angiotensin II formation with teprotide.

2. Infusion of noradrenaline by either route resulted in dose-related rises in plasma renin activity.

3. Pretreatment with teprotide reduced the rise in mean arterial pressure and abolished the rise in G.F.R. seen during intravenous infusions of noradrenaline (0.1, 0.2 and 0.4 µg/kg.min). Noradrenaline also reduced filtration fraction more after teprotide pretreatment.

4. Renal blood flow rose and renal vascular resistance fell in response to i.v. noradrenaline infusions. This renal vasodilatation was unaffected by pretreatment of the dogs with teprotide, indomethacin or DL-propranolol. However after pento- linium pretreatment, i.v. noradrenaline infusion caused a dose-related renal vasoconstriction.

5. Infusion of noradrenaline into the renal artery (0.02, 0.05 and 0.1 µg/kg.min) resulted in rises in mean arterial pressure and G.F.R. which were abolished by teprotide pretreatment. Filtration fraction rose when noradrenaline was administered alone but fell when it was infused after teprotide treatment.

6. Thus angiotensin II formed as the result of increased renin release acted to maintain G.F.R. and filtration fraction during noradrenaline infusion. In addition, i.v. noradrenaline infusions in conscious dogs caused reflex vasodilatation of the renal vasculature.

INTRODUCTION

Soon after techniques to measure glomerular filtration rate (G.F.R.) became available, it was observed that subcutaneously injected adrenaline reduced renal blood flow but increased filtration fraction (Chasis, Ranges, Goldring & Smith, 1938). Other investigators reported similar effects with both adrenaline (Barclay, Cooke & Kenney, 1947; Berne, Hoffman, Kagan & Levy, 1952; Jacobson, Hammarsten & Heller, 1951) and noradrenaline (Smythe, Nickel & Bradley, 1952; Pickford & Watt, 1951; Pullman & McClure, 1954) and it was thus suggested that these catecholamines primarily constricted the efferent renal arterioles, thereby increasing filtration fraction.
Subsequently these catecholamines were also shown to increase renin secretion (Vander, 1965). Since there is now strong evidence that angiotension II increases filtration fraction by constriction of efferent arterioles (Korner, Stokes, White & Chalmers, 1967; Regoli, 1972; Waugh, 1972; Hall, Guyton, Jackson, Coleman, Lohmeier & Trippodo, 1977), it seemed possible that angiotension II might mediate some of the effects of noradrenaline on renal blood flow and filtration fraction. To test this hypothesis, we have infused noradrenaline either intravenously or into the renal artery of conscious dogs and studied the effects of blockade of angiotensin II formation on the renal responses. Teprotide was used to block angiotension I converting enzyme and thus angiotension II formation. The involvement of circulatory reflexes, renal prostaglandins and β-adrenoceptors in the renal blood flow response to noradrenaline were also investigated.

METHODS

Animals

The experiments were performed in male mongrel dogs weighing from 19 to 30 kg (mean 25 kg). The dogs were trained to lie quietly on a padded table in the laboratory. Each dog then underwent surgery (under halothane and N₂O anaesthesia) in which one kidney was removed and a Doppler flow probe and an inflatable silastic cuff were positioned around the renal artery of the remaining kidney. Catheters (P.V.C.) were placed in the aorta and the inferior vena cava and also in the renal artery (in the dogs that received intrarenal infusions of noradrenaline) as previously described (Anderson, Johnston & Korner, 1979). Streptomycin (250 mg/day, i.m.) and penicillin (250 mg/day, i.m.) were given 1 week post-operatively. The dogs visited the laboratory daily for catheter flushing and were thoroughly accustomed to the laboratory as indicated by resting heart rates of about 70 beats/min.

Measurements

Phasic and mean aortic pressures were measured using a Statham P23DC transducer. Renal blood flow was measured by the Doppler ultrasonic flowmeter technique described previously (White, Angus, McGlache & Forges, 1974; Fletcher, Korner, Angus & Oliver, 1976; Anderson et al. 1979), with the units being kHz of Doppler frequency shift. In order to calculate filtration fraction, kH Doppler shift was converted to ml./min using a factor of 103 ml./min . kHz obtained in a separate series of calibration experiments. In these experiments, Doppler flow-meters were implanted around the carotid artery of these dogs and the arteries were then pump-perfused with blood at autopsy 2 weeks later. In two other dogs, renal artery Doppler flowmeters were pump-perfused in situ. The range for individual flowmeters was 95–108 ml./min . kHz, with a mean value of 103 ml./min . kHz.

Glomerular filtration rate was obtained from the renal clearance of [³H]inulin. Plasma and urine concentrations of [³H]inulin were determined by liquid scintillation spectrometry (200 µl. samples in 10 ml. liquid scintillation fluid (Insta-gel, Packard Instrument Company) were counted on a Packard Spectrometer, Model 3255). Use of [³H]inulin was validated in preliminary experiments in six anaesthetized dogs, in which the renal clearance of [³H]inulin was found to average 2.41 ± 0.31 compared to 2.42 ± 0.25 ml./min . kg body wt. for unlabelled inulin (Chicory root extract, Sigma). Renal plasma flow (R.P.F.) was calculated from renal blood flow by correcting for haematocrit, and filtration fraction was derived by G.F.R./R.P.F. Plasma renin activity was measured by radio-immunoassay (Anderson et al. 1979). The statistical significance of the various changes was assessed by two-way analysis of variance and orthogonal partitioning of the ‘between doses’ sums of squares (Snedecor & Cochran, 1967, pp. 308–310).

Protocols

In the first series of experiments noradrenaline was infused either intravenously or into the renal artery, with or without pretreatment with teprotide (n = 7). In these experiments a sterile bladder catheter (6 or 8 FG, Arnolds Veterinary Products Ltd, Reading) was advanced into the bladder. The dog was then placed on the padded experimental table. A 20 min period was allowed during which the aortic catheter and flowmeter were connected to a Devices recorder for blood pressure and renal blood flow measurements. A bolus infusion of [³H]inulin (1 µc/kg, i.v.) was then given followed by a maintenance infusion (1 µc/kg . hr, i.v.) allowing 40 min for equilibration. This was
followed by a 30 min control period. When appropriate, teprotide was administered (250 µg/kg, i.v. bolus, followed by a continuous infusion at 90 µg/kg . hr, i.v.) before a second 30 min control period. Teprotide at the dose used shifted the blood pressure response to i.v. bolus doses of angiotensin I to the right by 6–10 fold. L-noradrenaline tartrate (Sigma) dissolved in 0·9 % NaCl was infused intravenously at 0·1, 0·2, 0·4 µg/kg . min, for 40 min at each dose. Ten minutes was

Fig. 1. Average effects of i.v. infusions of noradrenaline at 0·1, 0·2, and 0·4 µg/kg . min with (interrupted lines) or without (continuous lines) teprotide pre-treatment. C1 = first control period, C2 = control period following teprotide (250 µg/kg bolus followed by 90 µg/kg . hr, i.v.). Bars at C1 are standard errors of means; bars at C2 are standard errors of differences from C1; bars at 0·2 µg/kg . min noradrenaline indicate the average errors for the three doses calculated from the analysis of variance error mean square (√(EMS/n) where n = no. of dogs).

allowed for steady-state conditions to occur and physiological changes were recorded for the last 30 min of each period. Seven dogs were each studied on three occasions: noradrenaline (i.v.) with and without teprotide and a control experiment in which 0·9 % NaCl solution was infused at equivalent rates to the noradrenaline infusions (i.e. 0·06, 0·15 and 0·30 ml./min). In six other dogs noradrenaline was infused into the renal artery at 0·02, 0·05 and 0·1 µg/kg . min with and without teprotide pretreatment.

In a separate series of experiments, noradrenaline was infused i.v. in four dogs pretreated with
one of the following: (i) pentolinium tartrate (6 mg/kg bolus, 3 mg/kg hr, i.v.), (ii) indomethacin (5 mg/kg, i.v.) dissolved in 40 ml phosphate buffer (0.187 % NaH2PO4, H2O, 0.754 % Na2HPO4) administered 1 hr before noradrenaline, (iii) DL-propranolol hydrochloride (1 mg/kg, 25 µg/kg min, i.v.) or (iv) phosphate buffer (40 ml). Dogs were allotted each procedure according to a Latin Square design. In these experiments, noradrenaline was infused at 0.02, 0.04, 0.01, 0.02 and 0.04 µg/kg min for 15 min each. G.F.R. was not measured in these experiments.

<table>
<thead>
<tr>
<th>Route of administration of noradrenaline</th>
<th>Dose of noradrenaline (µg/kg min)</th>
<th>Plasma renin activity (ng/ml hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td>Control</td>
<td>No pre-treatment 0.59 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.77 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>1.45 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>1.95 ± 0.34</td>
</tr>
<tr>
<td>Renal artery</td>
<td>Control</td>
<td>Teprotide 0.63 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>1.00 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>1.51 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>2.17 ± 0.62</td>
</tr>
</tbody>
</table>

**RESULTS**

**Effects of i.v. infusions**

Seven animals were studied on three occasions, receiving in random order either (i) noradrenaline (doses 0.1, 0.2, 0.4 µg/kg min, i.v.), (ii) noradrenaline at the above doses after teprotide pretreatment (250 µg/kg bolus, 90 µg/kg hr infusion) or (iii) 0.9 % NaCl. All significance levels given below are derived from two-way analysis of variance with appropriate orthogonal partitioning of the 'between doses' sums of squares. Teprotide had minimal effects on resting haemodynamics (Fig 1), causing small falls in mean arterial pressure (5 ± 1 mmHg, s.e.) and renal vascular resistance (9 ± 2 %). When the dogs were not given teprotide, arterial pressure did not change and renal vascular resistance rose (6 ± 3 %) over the same period (Fig 1). The effects of noradrenaline in each dog were compared to values in the second control period (C2) in each dog (Fig 1).

When noradrenaline was infused into dogs without teprotide pretreatment, mean arterial pressure increased as the dose increased, reaching +25 mmHg for 0.4 µg/kg min (P < 0.001, Fig 1). Surprisingly, noradrenaline caused renal blood flow to increase greatly with the level at the highest dose being 61 % greater than that for control (C2) (P < 0.001). Pretreatment with teprotide altered the magnitude of these responses to noradrenaline (Fig 1). Mean arterial pressure was on average 5 mmHg less than the corresponding levels when noradrenaline was infused alone (P < 0.025). Control renal blood flow was higher after teprotide and then increased in response to noradrenaline to the same extent as in the untreated dogs. Plasma renin activity was not significantly elevated at 0.1 µg/kg min (+0.18 ng/ml hr, Table 1) but rose significantly with respect to control at 0.4 µg/kg min (+1.36 ng/ml hr, P < 0.01). After teprotide, plasma renin activity rose more at each dose of noradrenaline (Table 1).
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Infusions of noradrenaline produced increases in glomerular filtration rate, averaging +20% at the highest dose \((P < 0.05)\). After teprotide pretreatment, there was no rise in glomerular filtration rate in response to noradrenaline. The lowest dose of noradrenaline decreased calculated filtration fraction in all dogs. At the two higher doses, filtration fraction rose back towards control in untreated dogs but fell progressively in teprotide-treated dogs \((P < 0.005)\). Control haematocrits were 38.0 and 38.1% in untreated and teprotide pretreated dogs respectively, and rose progressively to 45.5 and 46.8% respectively at the highest dose.

When 0.9% NaCl was infused in this group of dogs (Fig. 2), mean arterial pressure did not change and there were only small non-significant reductions in renal blood flow (total fall 13 ± 6%, s.e.) and G.F.R. (14 ± 10%).

**Analysis of renal haemodynamic responses to i.v. noradrenaline**

Noradrenaline was infused intravenously in four dogs after pretreatment with one of the following on different experimental days: (i) pentolinium tartrate; (ii) indomethacin (dissolved in phosphate buffer); (iii) DL-propranolol or (iv) control infusion of phosphate buffer. Noradrenaline infusion with phosphate buffer produced a dose-related increase in renal blood flow \((P < 0.005)\) and decrease in renal vascular resistance \((P < 0.01; \text{Fig. } 3)\). Noradrenaline infusions into pentolinium pretreated animals resulted in dose-related renal vasoconstriction. Indomethacin or DL-propranolol had no effect on the renal haemodynamic responses to noradrenaline. Plasma renin activity increased with each dose of noradrenaline in dogs which had received the control infusion (Table 2). The response in indomethacin pretreated
animals was not significantly different from the controls. Propranolol inhibited the renin response to noradrenaline \((P < 0.025,\) comparing the renin responses to noradrenaline with and without DL-propranolol pretreatment). Plasma renin activity increased less in pentolinium infused dogs than in the controls; however this effect was not significant.

Fig. 3. Average effects of noradrenaline infusions at 0.02–0.4 \(\mu\)g/kg.min, i.v. in dogs pretreated with pentolinium (squares), DL-propranolol (open circles), indomethacin (triangles), or buffer vehicle solution (thick line). Error bars indicate the within animal errors calculated from the analysis of variance error mean square \((\sqrt{\text{EMS}}/n)\) where \(n = \) no. of dogs).

Renal artery infusions of noradrenaline

In six dogs, noradrenaline was infused into the renal artery at the doses of 0.02, 0.05 and 0.1 \(\mu\)g/kg.min with or without teprotide pretreatment. Teprotide was administered 30 min before a control period. Noradrenaline caused a dose-related rise in mean arterial pressure \((+12 \text{ mmHg at } 0.1 \mu\text{g/kg.min in comparison to control, } P < 0.01)\). This pressor response was significantly reduced after teprotide pretreatment \((P < 0.01 \text{ when comparing the responses with or without teprotide, Fig. 4}).\) G.F.R. increased by an average of 26% from control at the highest dose of noradrenaline \((P < 0.05)\); teprotide pretreatment abolished this rise in g.f.r. (Fig. 4). Noradrenaline increased filtration fraction in the untreated dogs but reduced filtration fraction after teprotide pretreatment; the effect of teprotide on the filtration fraction responses to noradrenaline was significant \((P < 0.025)\).
TABLE 2. Plasma renin activity during intravenous infusion of noradrenaline alone or after pretreatment with indomethacin, propranolol or pentolinium (mean ± s.e.)

<table>
<thead>
<tr>
<th>Dose of noradrenaline (μg/kg . min)</th>
<th>Plasma renin activity (ng/ml . hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No treatment</td>
</tr>
<tr>
<td>Control</td>
<td>0.70 ± 0.17</td>
</tr>
<tr>
<td>0.02</td>
<td>0.70 ± 0.09</td>
</tr>
<tr>
<td>0.05</td>
<td>0.71 ± 0.10</td>
</tr>
<tr>
<td>0.1</td>
<td>1.21 ± 0.29</td>
</tr>
<tr>
<td>0.2</td>
<td>2.19 ± 0.53</td>
</tr>
<tr>
<td>0.4</td>
<td>3.57 ± 1.03</td>
</tr>
</tbody>
</table>

Fig. 4. Average changes in response to infusions of noradrenaline into the renal artery with tefrotide. Values are mean difference from control period (C). Tefrotide given before control period as 250 μg/kg bolus, followed by a constant infusion of 90 μg/kg . hr. Bars as for Fig. 3.

When noradrenaline was infused into the renal artery, renal blood flow again rose (P < 0.05) although the rise was not as great as when noradrenaline was infused intravenously. After tefrotide the increase in renal blood flow was smaller at all except the highest dose (P < 0.05). Renal vascular resistance did not change significantly from control for any of the doses used either with or without tefrotide. There were rises in plasma renin activity similar to those observed during i.v. administration (Table 1), and noradrenaline again resulted in greatly increased levels in activity after tefrotide pretreatment (Table 1).
Noradrenaline increased plasma renin activity during both i.v. and intrarenal infusions. The effects of angiotensin II generated by the increased plasma renin activity were studied by comparing the haemodynamic and glomerular filtration rate responses to noradrenaline in dogs with and without angiotensin I converting enzyme inhibition with teprotide.

The renin-angiotensin system played an important role in the glomerular filtration response to noradrenaline. When angiotensin II production was inhibited by teprotide, filtration fraction tended to be lower during noradrenaline infusion, suggesting that angiotensin II normally acted to maintain glomerular filtration rate. Such an action of angiotensin II is consistent with an effect on the efferent arterioles of the kidney in accord with previous studies (Korner et al. 1967; Regoli & Ganthier, 1971; Hall et al. 1977; Johns, Lewis & Singer, 1976). Although arterial levels of renin rose in this study, we cannot say whether the effects seen here were mediated by intravascularly or intrarenally generated angiotensin II. There is considerable evidence that angiotensin II can be formed intrarenally (Hofbauer, Zschiedrich & Gross, 1976; Merrill, Peach & Gilmore, 1973).

Angiotensin II also contributed to the rise in systemic arterial blood pressure during noradrenaline infusion. When noradrenaline was infused intrarenally, the generated angiotensin II accounted for all of the rise in arterial pressure: when noradrenaline was infused i.v. it accounted for about 20% of the rise.

A surprising finding was the increase in renal blood flow during intravenous infusion of noradrenaline. Our results indicate that this was due to a reflexly mediated vasodilatation, since noradrenaline caused renal vasoconstriction following blockade of efferent autonomic nerves with pentolinium. That is, increases in blood pressure during noradrenaline infusion reflexly inhibited renal sympathetic nerves, resulting in renal vasodilatation. This is in accordance with the findings of Iriki, Dorward & Korner (1977) who demonstrated in anaesthetized rabbits that an increase in blood pressure of 25 mmHg, similar to that reported here for the highest dose of noradrenaline, inhibited renal sympathetic tone by about 70%. Recently it has been suggested that the gain of this reflex may be even greater in conscious rabbits (Korner, Dorward, Blombery & Frean, 1980). Pentolinium caused a small fall in resting resistance in our conscious dogs (−9±6%) indicating the presence of some resting renal sympathetic tone although of low magnitude. This agrees with the findings of Korner et al. (1980) in conscious rabbits. In contrast, the renal vasodilatation was not mediated by β-adrenoceptor stimulation. Similarly we could find no role for prostaglandins in the renal blood flow response to noradrenaline in our conscious dogs in contrast to previous work in anaesthetized dogs (McGiff, Crowshaw, Terragno, Malik & Lonigro, 1972).

Teprotide pretreatment caused a fall in resting (pre-noradrenaline) renal vascular resistance and part of this response could be due to inhibition of kinin degradation in the kidney (Bakhle, 1968; Dorer, Kahn, Lentz, Levine & Skeggs, 1974; Clappison, Anderson & Johnston, 1980). Although it is possible that kinins are involved in the responses to noradrenaline, the increases in plasma renin activity suggest that angiotensin II is responsible for the effects.

Our results suggest that the so-called 'short loop' feed-back pathway (Davis &
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Freeman, 1976) of angiotensin II on renin release is very potent. The increases in plasma renin activity were about 5-fold greater when the dogs had been pretreated with teprotide. However other differences in the responses to noradrenaline may have contributed to the plasma renin activity differences (e.g. blood pressure, glomerular filtration rate, renal blood flow). Our results also agree with the suggestion that noradrenaline releases renin via a direct effect on β-adrenoceptors on the juxtaglomerular cells (Vandongen, Peart & Boyd, 1973; Assaykeen, Tanigawa & Allison, 1974). Propranolol completely blocked the rise in PRA during intravenous noradrenaline infusion at all doses except the highest (0.4 µg/kg.min). In contrast, prostaglandins were not involved in the release of renin in response to noradrenaline since pretreatment with indomethacin did not alter the response.

The direct effects of noradrenaline on renal glomerular function and haemodynamics appeared to be quite small. These effects are best seen in the dogs which received renal artery infusion of noradrenaline with teprotide pretreatment. In these dogs, in which increases in angiotensin II and arterial pressure did not occur, noradrenaline had minimal effects on filtration and renal blood flow. The absence of any blood pressure increases in these dogs also indicates that noradrenaline infused into the renal artery of these dogs was mainly removed from the circulation in the kidney and did not get to the rest of the body in significant amounts.

In summary, noradrenaline stimulated renin release from the kidney, mainly through β-adrenoceptor stimulation. The generated angiotensin II tended to maintain filtration fraction and also elevate arterial pressure, either accounting for all of the blood pressure elevation during renal artery infusions or part of the increase when noradrenaline was infused intravenously. This rise in blood pressure reflexly lowered renal vascular tone and blood flow increased.

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