Blood pressure response to chronic low-dose intrarenal noradrenaline infusion in conscious rats


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Summary

1. Sodium chloride solution (0.9%) or noradrenaline in doses of 4, 12 and 36 μg h⁻¹ kg⁻¹ was infused for five consecutive days, either intrarenally (by a new technique) or intravenously into rats with one kidney removed.

2. Intrarenal infusion of noradrenaline caused hypertension at doses which did not do so when infused intravenously.

3. Intrarenal compared with intravenous infusion of noradrenaline caused higher plasma noradrenaline concentrations and a shift of the plasma noradrenaline concentration–blood pressure effect curve towards lower plasma noradrenaline levels.

4. These results suggest that hypertension after chronic intrarenal noradrenaline infusion is produced by relatively higher levels of circulating noradrenaline and by triggering of an additional intrarenal pressor mechanism.

Key words: blood pressure, hypertension, intrarenal infusion, noradrenaline.

Introduction

The major conclusion of the systems analysis approach to blood pressure control [1] is that renal function dominates chronic blood pressure regulation. Chronic hypertension is due to a shift of the relationship between mean arterial pressure (MAP) and urine output, towards higher MAP. This shift in the pressure at which excretion of water and salt is enhanced by even small increases in MAP, is present in spontaneously hypertensive rats [2].

An increased sympathetic activity could initiate or maintain hypertension by influencing renal function and preventing pressure diuresis and natriuresis. Directly and indirectly induced sympathetic hyperactivity towards the kidney causes renal vasoconstriction [3, 4]. Additional neurogenic influences on renin release and renal tubular sodium reabsorption have been reviewed [5, 6]. Direct recording of renal nerve activity shows increases in sympathetic discharge frequency towards the kidney in spontaneously hypertensive rats [7] and renal denervation delays the onset of genetic hypertension and DOCA-salt hypertension in rats [8–10]. Furthermore, chronic intrarenal infusion of noradrenaline causes hypertension in dogs [11, 12].

Intrarenal infusion in conscious rats has not been achieved. In this paper, we present a new method for intrarenal application of drugs in conscious freely moving rats. We have used it to compare the effects of chronic intravenous and intrarenal infusion of several doses of noradrenaline on MAP and plasma noradrenaline concentrations in rats with one kidney removed. Part of the data in this paper have been presented previously in abstract form [13].

Methods

102 male Wistar rats (TNO, Zeist) varying in weight from 292 to 425 g were used. Four weeks after removal of the left kidney, the animals were prepared under ether anaesthesia for chronic infusion. In 59 rats the right suprarenal artery (Fig. 1) was cannulated with a stretched PE 10 catheter with
ultimate diameter 0.3 mm. This catheter (volume 12 μl) was guided backwards so that its tip reached the renal artery and it could be used for intrarenal application of drugs. A preliminary description of this surgery has been published recently [14]. In 43 rats, a catheter (volume 17 μl) was inserted into the right jugular vein for intravenous infusion. Catheters for intrarenal and intravenous infusion were flushed with sodium chloride solution (154 mmol/l: saline) from a subcutaneously implanted osmotic mini-pump (Alzet, type 2001: pumping rate approximately 1 μl/h).

In both groups of animals a catheter was inserted into the femoral artery for arterial pressure measurements and blood sampling. After this surgery, the animals were allowed to recover for 2 days. They were housed individually and had free access to food and water. Mean arterial pressure (MAP) was recorded under unrestrained conditions daily between 14.00 and 17.00 hours with a Statham P23Db strain gauge and a Grass 7P polygraph. During the last hour of each session, MAP was determined on a minute-to-minute basis and the average MAP was calculated. Only rats with differences between the 2 control days of less than 10% were used for infusion studies.

After this control period, saline minipumps were replaced under light ether anaesthesia by pumps containing either saline or noradrenaline solutions in such concentrations that rates of infusion of noradrenaline of 4 μg h⁻¹ kg⁻¹, 12 μg h⁻¹ kg⁻¹ and 36 μg h⁻¹ kg⁻¹ were obtained. Noradrenaline stays chemically stable inside the pumps in the presence of ascorbic acid (1 mg/ml) as was shown previously [15]. The infusions were continued for five consecutive days. Blood samples (0.5 ml) were taken on control days as well as on the first and fourth day after starting the infusions and were analysed for noradrenaline concentrations by means of a radioenzymatic assay [16]. Plasma noradrenaline levels during the intrarenal or intravenous infusion of the three doses of noradrenaline were plotted against corresponding blood pressures; log concentration-effect curves were fitted, a computer routine based on log-logit curve fitting as described by De Lean et al. [17] being used, running on a digital Minc 11 minicomputer. The goodness of fit was evaluated by chi-square test.

Further data were analysed statistically by means of non-parametric one-way analysis of variance for response curves [18] or a Student’s t-test for unpaired values.

Results

Intrarenal infusions of noradrenaline at all three doses significantly increased MAP after 5 days whereas intrarenal saline infusion did not (Student’s t-test; for P values, see Figures). Five days of intrarenal infusion of 4 μg of noradrenaline h⁻¹ kg⁻¹ (n = 14) increased MAP to 129 ± 4 mmHg (mean ± SEM); 12 μg h⁻¹ kg⁻¹ (n = 7) elevated MAP to 131 ± 3 mmHg and 36 μg h⁻¹ kg⁻¹ (n = 15) brought MAP up to 150 ± 3 mmHg (Fig. 2a).

Intravenous application at 4 μg of noradrenaline h⁻¹ kg⁻¹ (n = 8) for 5 days did not increase MAP. At 12 μg h⁻¹ kg⁻¹ (n = 10) the MAP increased significantly to 121 ± 3 mmHg at day 5 and with 36 μg h⁻¹ kg⁻¹ (n = 12) the MAP rose to 140 ± 4 mmHg on day 5 (Fig. 2b). Fig. 3 summarizes the results. Intrarenal noradrenaline infusion produced significantly greater increases in MAP than intravenous infusion at each dose throughout the infusion period. Control plasma noradrenaline levels before starting intrarenal infusions were 0.52 ± 0.01 ng/ml, and noradrenaline concentrations did not change during saline infusion when compared by means of a Student’s t-test. Intrarenal infusion of each dose of noradrenaline raised plasma noradrenaline concentrations slowly but significantly on day 4 (Fig. 4). Intrarenal infusion of 4 μg of noradrenaline h⁻¹ kg⁻¹ resulted in plasma noradrenaline levels of 1.65 ± 0.40 ng/ml, at doses of 12 μg of noradrenaline h⁻¹ kg⁻¹ plasma noradrenaline concentrations of 3.00 ± 0.60 ng/ml and at doses of 36 μg of noradrenaline h⁻¹ kg⁻¹ plasma noradrenaline levels of 7.33 ± 1.77 ng/ml were obtained.

On the day of starting intravenous infusions plasma noradrenaline concentrations were 0.56 ± 0.01 ng/ml; noradrenaline levels did not change significantly during chronic intravenous infusion of either saline or noradrenaline at doses of 4 μg h⁻¹ kg⁻¹. Doses of 12 μg of noradrenaline h⁻¹ kg⁻¹ raised plasma noradrenaline levels significantly to 1.61 ± 0.21 ng/ml, and doses of 36 μg of noradrenaline h⁻¹ kg⁻¹ to 3.63 ± 0.28 ng/ml on day 4. The role of the systemic elevations of plasma noradrenaline was further analysed by plotting plasma noradrenaline concentrations during intrarenal and
Intrarenal noradrenaline infusion in rats

Fig. 2. Effects on MAP during intrarenal (a) and intravenous (b) infusion of three doses of noradrenaline (●, 36 μg h⁻¹ kg⁻¹; ○, 12 μg h⁻¹ kg⁻¹; △, 4 μg h⁻¹ kg⁻¹), compared with the effects of saline (△). (*P < 0.05; **P < 0.005 (Student's t-test)).

Fig. 3. Differences in MAP during whole periods of intrarenal (●) and intravenous (○) infusion of three doses of noradrenaline [36(a), 12(b) and 4(c) μg h⁻¹ kg⁻¹] and saline (d). Arrows indicate first appearance of significant differences (P < 0.01, analysis of variance).

Intravenous noradrenaline infusion against corresponding blood pressures and fitting sigmoid log concentration–effect curves (Fig. 5). Goodness of fit was evaluated by applying the sum of square residues principle. Relationships between plasma noradrenaline concentrations and MAP values during intrarenal and intravenous noradrenaline infusions appeared to be described well enough (χ² = 28.62 < χ² P = 0.05 when ν = 40; χ² = 37.61 < χ² P = 0.05 when ν = 56) by presented log concentration–effect curves, indicating an increased responsiveness of MAP on elevation of plasma noradrenaline concentrations during intrarenal noradrenaline infusion vs intravenous noradrenaline infusion.

Discussion

Implantation of a stretched PE 10 catheter into the suprarenal artery [14] provides a new reliable
FIG. 4. Plasma noradrenaline concentrations during intrarenal (a) and intravenous (b) infusion of three doses of noradrenaline (4, 12 and 36 \( \mu \text{g h}^{-1} \text{kg}^{-1} \)) and saline (S). *\( P < 0.05 \); **\( P < 0.005 \) (Student's t-test).

FIG. 5. Comparison of log concentration-response curves during intrarenal (●) and intravenous (○) infusion of noradrenaline.
method for chronic intrarenal infusion of drugs in the conscious rat. Chronic intrarenal infusion of noradrenaline causes sustained hypertension at doses which are not effective intravenously and doses of noradrenaline which raise the MAP when given intravenously cause even greater increases in MAP when given intrarenally. These findings support the hypothesis that a renal neurogenic factor, e.g. intrarenal noradrenergic activity, is involved in the development and maintenance of experimental hypertension in rats [7-10] and essential hypertension in human [19]. A similar observation was made in one-kidney normotensive conscious dogs, where chronic intrarenal noradrenaline infusion at a rate of 17 µg h⁻¹ kg⁻¹ caused a sustained elevation in MAP of approximately 25 mmHg, and intravenous infusion of the same dose resulted in either transient [11] or sustained but significantly smaller increases in blood pressure [12]. However, plasma noradrenaline levels during intravenous and intrarenal infusion appeared to be the same at day 1 [11]. We found higher increases in plasma noradrenaline during intrarenal noradrenaline infusion, especially on day 4 (Fig. 4), which means that at least part of the observed higher MAP during intrarenal noradrenaline infusion can be attributed to higher plasma noradrenaline concentrations.

Either differences in modulation of the endogenous noradrenaline release mechanism or relatively more decreased noradrenaline clearance can account for higher plasma noradrenaline levels during intrarenal infusion. Circulating noradrenaline is mainly cleared by re-uptake in sympathetic nerve endings (uptake₁) [20] and blood flow to richly innervated organs could determine noradrenaline clearance. Higher plasma levels of noradrenaline during intrarenal noradrenaline infusion could be caused by differences in blood flow towards clearing organs, resulting from alterations in plasma volume regulation during intrarenal noradrenaline infusion. However, plasma volume or blood flow distribution data during chronic intrarenal noradrenaline infusion in the dog or in the rat are not available yet.

It could be argued that the higher increases in MAP as well as in plasma noradrenaline concentrations during intrarenal noradrenaline infusion are the consequence of noradrenaline-induced renal insufficiency. Both glomerular filtration and tubular secretion contribute to the renal clearance of unbound noradrenaline [21]. It is an assumption that the concentration of unbound noradrenaline in the kidney is much higher during intrarenal than during intravenous noradrenaline infusion. It is possible that the decrease in GFR to 70% of control values observed during intrarenal infusion of 36 µg of noradrenaline h⁻¹ kg⁻¹ [22] results in a decreased renal noradrenaline clearance and at least partly explains the higher plasma noradrenaline levels.

An alternative explanation for the higher elevations in plasma noradrenaline concentration during intrarenal noradrenaline infusion could be a relatively more activated sympathetic nerve system, due to stimulation of renal afferent nerves. Katholi et al. [23] proposed a similar role for the sympathetic nerve system in established two-kidney, one-clip Goldblatt hypertension.

Intrarenal infusion of noradrenaline at doses as low as 4 µg h⁻¹ kg⁻¹ for 24 h caused hypertension without a detectable increase in the plasma noradrenaline levels. The increased responsiveness of MAP to the plasma noradrenaline levels during intrarenal infusion of three doses of noradrenaline was assessed by plotting plasma noradrenaline concentrations against corresponding MAP values and fitting log concentration-effect curves. The lack of plasma noradrenaline data lying on the flat upper part of the concentration-effect curve during intravenous noradrenaline infusion does not necessarily lead to misinterpretation, since the log-logit curve-fitting computer model is able to correct for this problem [17]. It appears that the log concentration-effect curve is shifted to the left during intrarenal noradrenaline infusion vs intravenous infusion, indicating that any elevation of plasma noradrenaline levels above approximately 1.40 ng/ml caused by intrarenal noradrenaline infusion induces higher levels of MAP, compared with the effects on MAP during intravenous infusion.

This extra pressor effect during intrarenal noradrenaline infusion could be explained by increased sensitivity of the noradrenaline receptors, which are related to the rise in blood pressure. This is possibly caused by varied modulation of electrolyte balance, e.g. on the level of tubular sodium reabsorption [6], which has been shown to mediate noradrenaline-receptor sensitivity in the dog [12]. However, another explanation could be triggering of an additional intrarenally located pressor mechanism such as activation of the renin-angiotensin system. Increased plasma renin activity (PRA) during intrarenal noradrenaline infusion in the dog has been reported [12, 24]. Chronic increases in PRA elevate circulating angiotensin II levels, which in turn result in hypertension by the direct pressor effects [25].

Furthermore, angiotensin II facilitates noradrenaline release by adrenergic nerve endings [26], which could explain the higher plasma noradrenaline levels during intrarenal infusion of noradrenaline.
References


