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# Blood Pressure, the Renin-angiotensin System and Neurogenic Vasoconstriction in Pithed Rats

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**Abstract**—The influence of changing baseline blood pressure by various means, both related or unrelated to the renin-angiotensin system, on the pressor responses to spinal cord stimulation has been examined in the pithed rat. Mean arterial pressure and neurogenic vasoconstriction were higher in pithed rats with intact kidneys (2-kidney rats) than in nephrectomized pithed rats. Increasing blood pressure by infusion of vasopressin increased the pressor response to nerve stimulation in both 2-kidney and nephrectomized pithed rats. Decreasing blood pressure produced by administration of enalaprilat or hydralazine in 2-kidney pithed rats or by administration of hydralazine in nephrectomized pithed rats, decreased the pressor responses to nerve stimulation. Our results showed a positive correlation between the mean arterial blood pressure and the response to nerve stimulation in pithed rats. Therefore, we conclude that the pithed rat is an animal model which should be used with caution to study the interaction between the sympathetic nervous system and drugs which change baseline blood pressure.

It has been proposed that angiotensin II potentiates the activity of the peripheral sympathetic nervous system (Zimmerman et al 1984; Story & Ziogas 1987). This proposition is partly based on the observation that pharmacological (angiotensin converting enzyme inhibitors and angiotensin II antagonist) or surgical (nephrectomy) interruption of the renin-angiotensin system decreases the pressor response following stimulation of the lumbar sympathetic outflow in pithed rats (Antonaccio & Kerwin 1981; Clough et al 1982; De Jonge et al 1983; Hatton & Clough 1982; Vollmer et al 1984; Kaufman & Vollmer 1985; Atkinson et al 1987). Moreover, the attenuation of the pressor response is reversed after infusion of exogenous angiotensin II. However, restoration of pressor responsiveness could also be obtained by infusion of another pressor agent, vasopressin (Johnson et al 1974; De Jonge et al 1983; Oldham & Scotcher 1985; Vollmer et al 1988) which, in contrast with angiotensin II, has not been shown to potentiate the release of noradrenaline from sympathetic nerve terminals (Starke et al 1970; Hughes & Roth 1971; Johnson et al 1974; Starke 1981). These observations indicate that the baseline blood pressure may be a determinant of the pressor responsiveness in pithed rats. Thus angiotensin II may influence pressor responses to stimulation of lumbar sympathetic outflow in pithed rats by its effects on baseline blood pressure.

The present study was designed to investigate further the interaction between the renin-angiotensin system, the sympathetic nervous system, and blood pressure in the pithed rat. We studied the effect of changing baseline blood pressure by drugs related or unrelated to the renin-angiotensin system, on the pressor responses following electrical stimulation of the lumbar sympathetic outflow.

## Materials and Methods

Male Sprague-Dawley rats, 280–330 g, had free access to food and water. Studies were done in rats with intact kidneys (2-kidney) and nephrectomized animals. Bilateral nephrectomy was performed under diethylether anaesthesia 18 to 24 h before the experiment. The kidneys were exposed retroperitoneally via bilateral flank incisions. The capsule was carefully removed and care taken to avoid damage to the adrenal glands. The renal artery, vein, and ureter were ligated and the kidney removed.

Pithed rats were prepared according to Gillespie & Muir (1967). During a short period of anaesthesia with diethylether, the trachea was cannulated and the animals were pithed with a stainless steel rod passed through the orbit of the right eye. Immediately after pithing, the rats were ventilated with room air enriched with 33% oxygen using a respiratory pump (Harvard model 683, South Natick, MASS, USA at 50 cycles $min^{-1}$, 10 mL $kg^{-1}$). The body temperature of the rat was maintained at 37°C, by a thermostatically-controlled heating lamp, monitored with a rectal thermometer (Systag TCU-82, Rüslikon, Switzerland).

The left common carotid artery was cannulated for blood pressure measurement (Isotec transducer, Miamisbourg, OH, USA; Hellige recorder, Freiburg im Breisgau, Germany). Drugs were administered through a cannula placed in the right jugular vein. Atropine 0·3 mg $kg^{-1}$ and tubocurarine 1 mg $kg^{-1}$ were given intravenously immediately after pithing the animal. Blood gases and pH were measured in samples (0·2 mL) collected from the carotid artery cannula, using a blood gas analyser (Radiometer ABL3, Copenhagen, Denmark). Values were $Po_2 > 90$ mmHg, $Pco_2 = 30$–40 mmHg and pH 7·4–7·5.

Pressure responses to electrical stimulation were measured in 2-kidney and nephrectomized animals. After a 15 min equilibration period following the pithing procedure, electrical stimulation was induced through the pithing rod for 30 s, at 5 min intervals, using a supramaximal voltage of 60V, 2 ms impulses and 2 frequencies, 0·3 or 1 Hz (Grass S44 stimulator, Quincy. Mass., USA). After an additional 15 min period, either 0·15 M saline, enalaprilat 3 mg $kg^{-1}$, hydralazine 0·3 mg $kg^{-1}$ or arginine-vasopressin 0·1 mg $kg^{-1}$ $min^{-1}$

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were administered i.v. and the blood pressure was allowed to stabilize for 15 min before a second series of stimulations was performed. Within 2-kidney and nephrectomized pithed rats, there were no significant differences between groups for preinjection values of mean arterial blood pressure and pressor responses to electrical stimulation.

*Drugs*

All drugs were administered in NaCl 0·15 M. Drugs used were arginine-vasopressin (Bachem, Budendorf, Switzerland), atropine sulphate (Sigfried, Zofingen, Switzerland), hydralazine (Apresolin, Ciba-Geigy, Basel, Switzerland), enalaprilat (MK422, MSD, Rahway, USA), tubocurarine hydrochloride (Tubarine, Wellcome, London, UK).

*Statistical analysis*

All values given in the text and figures are mean ± s.e.mean. The statistical significance between groups was assessed by unpaired Student's *t*-test. Correlation coefficients were obtained by linear regression analysis of pooled data.

## Results

Before any injection, mean arterial blood pressure (MAP) and the pressor responses to electrical stimulation at 0·3 or 1 Hz were significantly lower in nephrectomized pithed rats than in 2-kidney pithed rats (Table 1). Injection of saline had no effect on MAP or on the pressor responses to electrical stimulation in 2-kidney or nephrectomized pithed rats (Table 1).

Treatment of 2-kidney pithed rats with the converting enzyme inhibitor enalaprilat induced a fall in MAP, and a decrease in the responses to nerve stimulation (Fig. 1, left panel). In nephrectomized pithed animals, enalaprilat had no significant effect on either MAP or the response to electrical stimulation (Fig. 1, right panel).

In contrast, hydralazine lowered MAP and the response to nerve stimulation, in both 2-kidney and nephrectomized pithed rats. Arginine-vasopressin increased MAP and the response to nerve stimulation in both 2-kidney and nephrectomized pithed rats (Fig. 1).

When the data from all these experiments were pooled, there was a significant positive correlation between the baseline blood pressure and the responses to electrical stimulation at 0·3 Hz (Fig. 2A) and 1 Hz (Fig. 2B). The equations of the best fit line were $\Delta BP = 0{\cdot}75\,MAP - 24{\cdot}81$ at 0·3 Hz and $\Delta BP = 1{\cdot}26\,MAP - 29{\cdot}16$ at 1 Hz, where MAP represents the baseline blood pressure and ΔBP the increase in blood pressure induced by electrical stimulation. The correlation coefficients were r = 0·789 at 0·3 Hz ($P < 0{\cdot}001$); r = 0·741 at 1 Hz, ($P < 0{\cdot}001$).

Table 1. Mean arterial blood pressure (MAP, mmHg) and pressor responses to electrical stimulation at 0·3 and 1·0 Hz (ΔBP, mmHg) in 2-kidney (NR) and nephrectomized pithed rats (NXR), before and after administration of saline. Significant differences between 2-kidney and nephrectomized pithed rats before and after saline were determined by unpaired Student's *t*-test: *$P<0{\cdot}05$; **$P<0{\cdot}01$; ***$P<0{\cdot}001$. For these two groups, n is 12.

| | Before saline | | After saline | |
|---|---|---|---|---|
| | NR | NXR | NR | NXR |
| MAP | 67·5 ± 3·8 | 42·8 ± 2·0*** | 68·3 ± 3·8 | 43·9 ± 1·9*** |
| ΔBP 0·3 Hz | 28·0 ± 4·0 | 4·7 ± 1·0*** | 30·8 ± 4·3 | 4·6 ± 0·8*** |
| ΔBP 1·0 Hz | 63·5 ± 4·9 | 18·8 ± 3·0*** | 63·9 ± 4·4 | 17·9 ± 2·6*** |

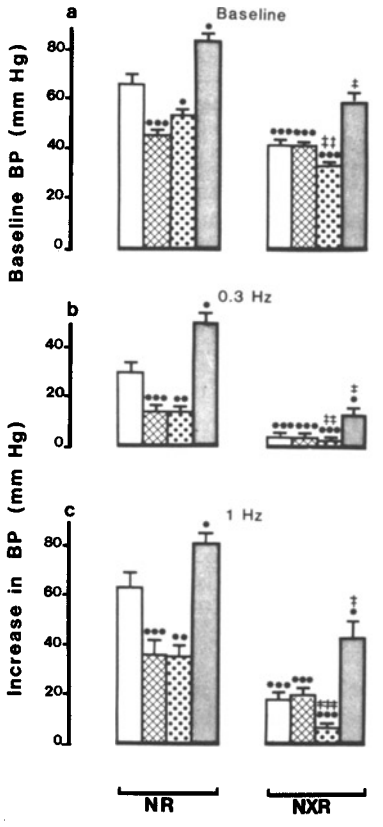


FIG. 1. Mean arterial blood pressure (a), and increase in blood pressure induced by electrical stimulation at 0·3 Hz (b) and 1 Hz (c) in 2-kidney (NR) (left panel) and nephrectomized pithed rats (NXR) (right panel), after administration of 0·15 M saline (open bar), enalaprilat 3 mg $kg^{-1}$ (cross-hatch bar), hydralazine 0·3 mg $kg^{-1}$ (spotted bar), or arginine-vasopressin 0·1 mg $kg^{-1}$ (open bar). Vertical lines depict s.e. mean. Significant differences between the saline group in 2-kidney pithed rats and the other groups were determined by unpaired Student's *t*-test: *$P<0{\cdot}05$; **$P<0{\cdot}01$; ***$P<0{\cdot}001$.

In nephrectomized pithed rats, significant differences between saline group and the drug groups were determined by unpaired Student's *t*-test: ‡$P<0{\cdot}05$; ‡‡$P<0{\cdot}01$; ‡‡‡$P<0{\cdot}001$. For all groups, n is at least 10.

## Discussion

Our results show a close relationship between baseline MAP and pressor responses to low frequency (0·3 or 1 Hz) stimulation of sympathetic nerve outflow in pithed rats. Decreases in baseline MAP produced by a variety of procedures [nephrectomy, a converting enzyme inhibitor (enalaprilat), or a vasodilator (hydralazine)] were all accompanied by a decrease in pressor responses to stimulation of sympathetic outflow. An increase in baseline MAP produced by infusion of vasopressin (in both 2-kidney and nephrectomized rats) was accompanied by an increase in pressor responses to stimulation of sympathetic outflow.

Early work suggested that interruption of the renin-angiotensin system following administration of the converting enzyme inhibitor, captopril, was the cause of the decrease

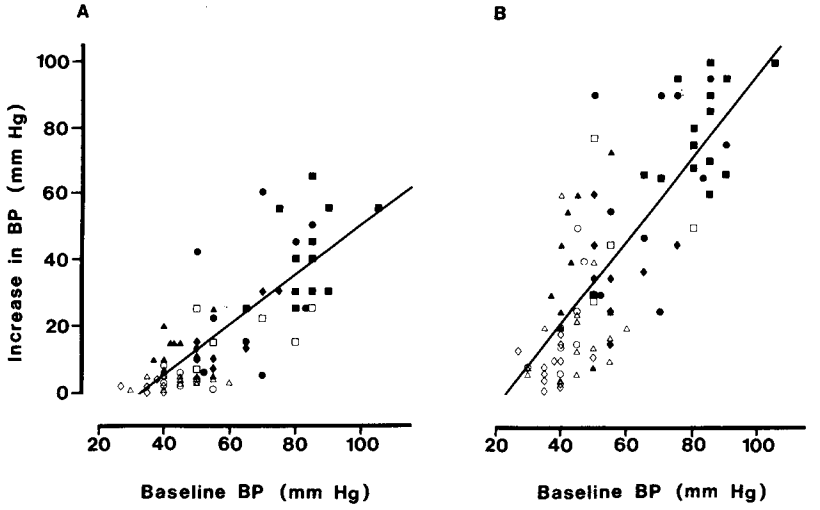


FIG. 2. Correlations between baseline blood pressure and responses to nerve stimulation (Fig. 2A: 0·3 Hz; Fig. 2B: 1 Hz) Symbols are for 2-kidney rats: saline (●), enalaprilat (▲), hydralazine (◆), arginine-vasopressin (■); and for nephrectomized rats: saline (○), enalaprilat (△), hydralazine (◇), arginine-vasopressin (□). The line of best fit was obtained by linear regression analysis.

in pressor responsiveness seen in pithed rats (Clough et al 1982). However, captopril also produces a fall in baseline MAP in this model. Moreover if baseline MAP is lowered by unrelated procedures such as administration of sodium nitroprusside or the potassium channel opener, cromakalim, or by haemorrhage, pressor responsiveness is also attenuated (Oldham & Scotcher 1985; Buckingham 1988; Grant & McGrath 1988a). Since both vasodilators and haemorrhage stimulate the renin-angiotensin system whereas captopril inhibits this system, it would appear that the common factor behind the effect of these procedures on pressor responsiveness is not interruption of the renin-angiotensin system but in the fall in baseline MAP which they produce. This hypothesis is confirmed by our results and is also supported by Grant & McGrath (1988a, b) who showed that falls in baseline MAP produced by inhibitors of the renin-angiotensin system (teprotide and saralasin) or sodium nitroprusside were followed by a decrease in the pressor response to electric stimulation.

In our experiments increases in baseline MAP by vasopressin in both nephrectomized and non-nephrectomized rats were accompanied by increases in pressor responsiveness. These findings confirm those reported by De Jonge et al (1983) who showed that restoration of baseline MAP by vasopressin in captopril-treated rats led to a recovery of pressor responsiveness.

In conclusion, the present results support the hypothesis that the action of converting enzyme inhibitors and angiotensin II in the pithed rat may be due to a change in basal arteriolar muscular tone rather than to a direct effect of angiotensin II on sympathetic nerve activity. Furthermore, in the pithed rat, vasoactive drugs with different mechanisms of action can change pressor responses to sympathetic nerve stimulation simply by altering baseline blood pressure values. Therefore, in such an animal model, conclusions on the mechanism of action of drugs which change baseline vascular tone should be drawn carefully. Earlier studies on the interaction between the peripheral sympathetic nervous system and the renin-angiotensin system in pithed rats should be reinterpreted with caution.

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## References

Antonaccio, M. J., Kerwin, L. (1981) Pre- and postjunctional inhibition of vascular sympathetic function by captopril in SHR. Hypertension 3(suppl I): I-54-I-62

Atkinson, J., Sonnay, M., Sautel, M., Fouda, A. K. (1987) Chronic treatment of the spontaneously hypertensive rat with captopril attenuates responses to noradrenaline in vivo but not in vitro. Naunyn-Schmiedeberg's Arch. Pharmacol. 335: 624–628

Buckingham, R. E. (1988) Studies on the anti-vasoconstrictor activity of BRL 34915 in spontaneously hypertensive rats; a comparison with nifedipine. Br. J. Pharmacol. 93: 541–552

Clough, D. P., Collis, M. G., Conway, J., Hatton, R., Keddie, J. R. (1982) Interaction of angiotensin-converting enzyme inhibitor with the function of sympathetic nervous system. Am. J. Cardiol. 49: 1410–1414

De Jonge, A., Knape, J. Th. A., Van Meel, J. C. A., Kalkman, H. O., Wilffert, B., Thoolen, M. J. M. C., Van Brummelen, P., Timmermans, P. B. M. W. M., Van Zwieten, P. A. (1983) Effect of captopril on sympathetic neurotransmission in pithed normotensive rats. Eur. J. Pharmacol. 88, 231–240

Gillespsie, J. S., Muir, T. C. (1967) A method of stimulating the complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat. Br. J. Pharmacol. 30: 78–87

Grant, T. L., McGrath, J. C. (1988a) Interactions between angiotensin II, sympathetic nerve-mediated pressor response and cyclooxygenase products in the pithed rat. Ibid. 95: 1220–1228

Grant, T. L., McGrath, J. C. (1988b) Interactions between angiotensin II and alpha-adrenoceptor agonists mediating pressor responses in the pithed rat. Ibid. 95: 1229–1240

Hatton, R., Clough, D. P. (1982) Captopril interferes with neurogenic vasoconstriction in the pithed rat by angiotensin-dependent mechanisms. J. Cardiovasc. Pharmacol. 4: 116–123

Hughes, J., Roth, R. H. (1971) Evidence that angiotensin enhances transmitter release during sympathetic nerve stimulation. Br. J. Pharmacol. 41: 239–255

Johnson, E. M. Jr, Marshall, G. R., Needleman, P. (1974) Modification of responses to sympathetic nerve stimulation by the renin-angiotensin system in rats. Ibid. 51: 541–547

Kaufman, L. J., Vollmer, R. R. (1985) Endogenous angiotensin II facilitates sympathetically mediated hemodynamic responses in pithed rats. J. Pharmacol. Exp. Ther. 235: 128–134

Oldham, A. A., Scotcher, K. P. (1985) Interactions between angiotensin II, the sympathetic nervous system, and blood pressure of the pithed rat. Br. J. Pharmacol. 86: 749P

Starke, K. (1981) Regulation of noradrenaline release by presynaptic receptor systems. Rev. Physiol. Biochem. Pharmacol. 77: 1–124

Starke, K., Werner, U., Hellerforth, R., Schumann, H. J. (1970) Influence of peptides on the output of noradrenaline from isolated rabbit hearts. Eur. J. Pharmacol. 9:136–140

Story, D. F., Ziogas, J. (1987) Interaction of angiotensin with noradrenergic neuroeffector transmission. TIPS 8:269–271

Vollmer, R. R., Meyers, S. A., Ertel, R. J., Murthy, V. S. (1984) Diminished sympathetic responsiveness in nephrectomized rats—Role of the renin angiotensin system. Clin. and Exper.—Theory and Practice A6(5): 993–1009

Vollmer, R. R., Corey, S. P., Fluharty, S. J. (1988) Angiotensin II facilitation of pressor responses to adrenal field stimulation in pithed rats. Am. J. Physiol. 254: R95–R101

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