*Br. J. Pharmac.* (1985), **85**, 897–903

# The influence of neonatal treatment with capsaicin on the control of blood pressure in adult rats in water-replete and water-deprived states

T. Bennett & S.M. Gardiner

Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Clifton Boulevard, Nottingham NG7 2UH

1 Arterial blood pressures and heart rates were measured in water-replete and in water-deprived (48 h) conscious, adult rats that had received capsaicin (50 mg $kg^{-1}$) or its vehicle neonatally.

2 Resting arterial blood pressures and heart rates in capsaicin-treated rats were not different from the controls in either the water-replete or the water-deprived state.

3 Inhibition of the vascular actions of vasopressin (with 1-β-mercapto,-β,β-cyclopentamethylenepropionic acid, 8-D-arginine vasopressin, (d($CH_2$)$_5$DAVP)) had no significant effect on blood pressures in the water-replete animals but caused a significant hypotension in water-deprived rats; the magnitude of the hypotension was the same irrespective of whether the animals had received capsaicin or its vehicle.

4 During angiotensin converting enzyme inhibition (with captopril) and ganglion blockade (with pentolinium), the vasopressin-mediated blood pressure recovery was more gradual in the capsaicin-treated animals than in the controls, but after 60 min blood pressures were similar in all groups. Collectively the results indicate that although the full development of vasopressin-dependent mechanisms following acute hypotension takes longer when a large proportion of unmyelinated afferent fibres have been destroyed by neonatal treatment with capsaicin, 48 h of water deprivation results in a normal involvement of vasopressin-dependent mechanisms in the maintenance of blood pressure.

## Introduction

Treatment of 2 day old rats with an appropriate dose of capsaicin results in a selective and permanent destruction of up to 90% of peripheral unmyelinated afferent fibres (Nagy *et al.*, 1981). Unmyelinated afferent fibres may be involved in the reflex release of vasopressin in response to non-osmotic stimuli (Thoren, 1979), and consistent with this, we recently showed impaired vasopressin-mediated blood pressure recovery following acute hypotension in water-deprived, adult Wistar rats which had received capsaicin neonatally (Bennett & Gardiner, 1984). In our previous study, we commented on the fact that fluid balance (as judged by body weight loss) in capsaicin-treated animals was not different from that of the control animals during water deprivation. Since, *in vivo*, the threshold for the overt cardiovascular effects of vasopressin is reported to be much higher than that for its renal effects (Cowley *et al.*, 1983), it is possible that impaired vasopressin release in animals treated neonatally with capsaicin might not be manifest as an inability to control fluid balance, but might be apparent as a diminished contribution by vasopressin to the maintenance of blood pressure during water deprivation. The aim of the present study was, therefore, to determine the contribution from vasopressin and the renin-angiotensin system to the maintenance of blood pressure in capsaicin-treated rats and vehicle-injected animals in the water-deprived state; for comparison, we performed the same experiment in water-replete animals.

## Methods

Wistar rats were anaesthetized with halothane (in oxygen) on day 2 after birth and injected subcutaneously with either vehicle (10% ethanol, 10% Tween 80 in 0.9% NaCl) or capsaicin (50 mg $kg^{-1}$) in a volume of 100 μl. After weaning (day 21–30 after birth) the animals were housed in groups of 4 or 5 with

free access to food (Labsure 41B; 0.06 mmol $g^{-1}$ $Na^+$, 0.14 mmol $g^{-1}$ $K^+$; 4% water) and tap water until the time of the study 18–20 weeks later.

Twelve groups of animals ($n = 6$ in each group) were used. Six groups (3 capsaicin-treated, 3 vehicle-injected) were deprived of drinking water, but not food for the 48 h before the experiment; the remaining groups had free access to water throughout.

On the day of the experiment animals were anaesthetized with a short-acting barbiturate (sodium methohexitone, 60 mg $kg^{-1}$, i.p.). Blood was obtained by cardiac puncture from 4 groups of animals (vehicle-injected and capsaicin-treated, water-replete and water-deprived) and measurements were made of plasma sodium and potassium by flame photometry (Instrumentation Laboratory 943) and osmolality by freezing point depression (Advanced Digimatic Osmometer, 3D II). In the 8 groups of animals in which cardiovascular studies were carried out, blood samples were not taken.

Three catheters were implanted in the jugular vein (for drug administration) and 1 catheter was introduced into the abdominal aorta via the caudal artery (for blood pressure and heart rate recordings). The catheters were led subcutaneously to the dorsal cervical region where they were exteriorised and then passed through a flexible protective spring attached to a counter-balanced support system. Details of the catheter and recording devices are given elsewhere (Gardiner *et al.*, 1980). The experiments began 5 h after surgery when the unrestrained animals were fully conscious. By this time the animals are in a steady state (Gardiner *et al.*, 1980) and even after more extensive surgery than we performed, plasma vasopressin levels are normal (Rascher *et al.*, 1983). The animals showed no signs of discomfort.

Two protocols were used: in 4 groups (water-replete and water-deprived; capsaicin-treated and vehicle-injected), administration of an antagonist of the actions of vasopressin mediated through $V_1$-receptors (Manning *et al.*, 1982; 1-β-mercapto,-β,β-cyclopentamethylene propionic acid, 8-D-arginine vasopressin ($d(CH_2)_5DAVP$); 10 μg $kg^{-1}$ bolus; 6 μg $kg^{-1}$ $h^{-1}$ infusion) was followed 1 h later by pentolinium (5 mg $kg^{-1}$ bolus; 3 mg $kg^{-1}$ $h^{-1}$ infusion) and finally captopril (2 mg $kg^{-1}$ bolus; 0.6 mg $kg^{-1}$ $h^{-1}$ infusion).

In the other 4 groups the order of $d(CH_2)_5DAVP$ and captopril administration was reversed.

*Drugs*

Bolus injections were given in a volume of 100 μl and the infusion rate was 0.3 ml $h^{-1}$. The doses of captopril and $d(CH_2)_5DAVP$ were sufficient to abolish the pressor effects (50–60 mmHg) of 125 ng of angiotensin I and of 5 mu of vasopressin respectively for the duration of the experiment. It is possible that differences in volumes of distribution and pharmacokinetics could give rise to higher plasma concentrations of drugs in the water-deprived, compared to the water-replete, states, and it could be argued that this was the explanation for the different resonses to the drugs described in the results. In order to be assured that the doses of captopril and $d(CH_2)_5DAVP$ used were exerting a maximal effect on the processes we were seeking to inhibit, in a separate experiment on 2 fluid-replete control rats, we increased the doses of captopril and $d(CH_5)_5DAVP$ 10 fold. These doses, like the lower doses, had no hypotensive effect. Furthermore, under all experimental conditions, in the presence of all three drugs, there were no signs of residual pressor activity, thus indicating that the cardiovascular effects of vasopressin and the renin-angiotensin system were abolished by the doses of $d(CH_2)_5DAVP$ and captopril we employed (see results).

The drugs used were pentolinium tartrate (May & Baker), captopril (Squibb), arginine vasopressin (Cambridge Biochemicals), angiotensin I (Sigma), $d(CH_2)_5DAVP$ (1-β-mercapto,-β,β-cyclopentamethylenepropionic acid, 8-D-arginine vasopressin; from Professor M. Manning, Ohio).

*Statistics*

Values are expressed as the mean ± s.e.mean; $n$ is the number of animals. Results were analysed for statistical significance by the Mann Whitney U-test (unpaired) or Wilcoxon rank sum test (paired) as appropriate.

## Results

The results obtained in male and female rats were not different and were therefore combined. In the water-replete state, vehicle- and capsaicin-treated animals had similar plasma sodium (144 ± 0.5; 145 ± 0.5 mmol $l^{-1}$ respectively) and potassium levels (4.7 ± 0.1; 4.9 ± 0.1 mmol $l^{-1}$ respectively). However, in these groups, plasma osmolality was slight higher in capsaicin-treated (305 ± 2 mosm $kg^{-1}$) than in vehicle-injected (299 ± 2 mosm $kg^{-1}$; $P < 0.05$) animals. Following 48 h water deprivation both groups had similar plasma sodium (capsaicin = 149 ± 0.03mmol $l^{-1}$; vehicle = 148 ± 0.6 mmol $l^{-1}$) and potassium levels (capsaicin = 4.5 ± 0.1 mmol $l^{-1}$; vehicle = 4.2 ± 0.1 mmol $l^{-1}$) and osmolalities (capsaicin = 312 ± 3 mosm $kg^{-1}$; vehicle ± 309 ± 2 mosm $kg^{-1}$), and had lost amounts in body weight that were not significantly different (capsaicin = 33 ± 3g; vehicle = 26 ± 4g).

Resting arterial blood pressures in water-replete, vehicle-injected rats (169 ± 2/101 ± 3 mmHg; systolic/diastolic; $n = 12$) were not different from those of

water-replete, capsaicin-treated animals (167 ± 2/105 ± 2 mmHg; $n = 12$). Similarly, in the water-deprived state, resting arterial blood presures in the 2 groups were not different (vehicle = 175 ± 2/109 ± 1 mmHg; $n = 12$; capsaicin = 171 ± 2/109 ± 1 mmHg; $n = 12$). In the vehicle-injected rats only, diastolic blood pressures were significantly higher ($P < 0.02$) in the water-deprived than in the water-replete state.

Resting heart rates in the 2 groups of water-replete rats were not different (vehicle = 372 ± 8, capsaicin = 362 ± 5 beats $min^{-1}$). The 2 groups of water-deprived rats also had similar resting heart rates (vehicle = 381 ± 5, capsaicin = 381 ± 6 beats $min^{-1}$).

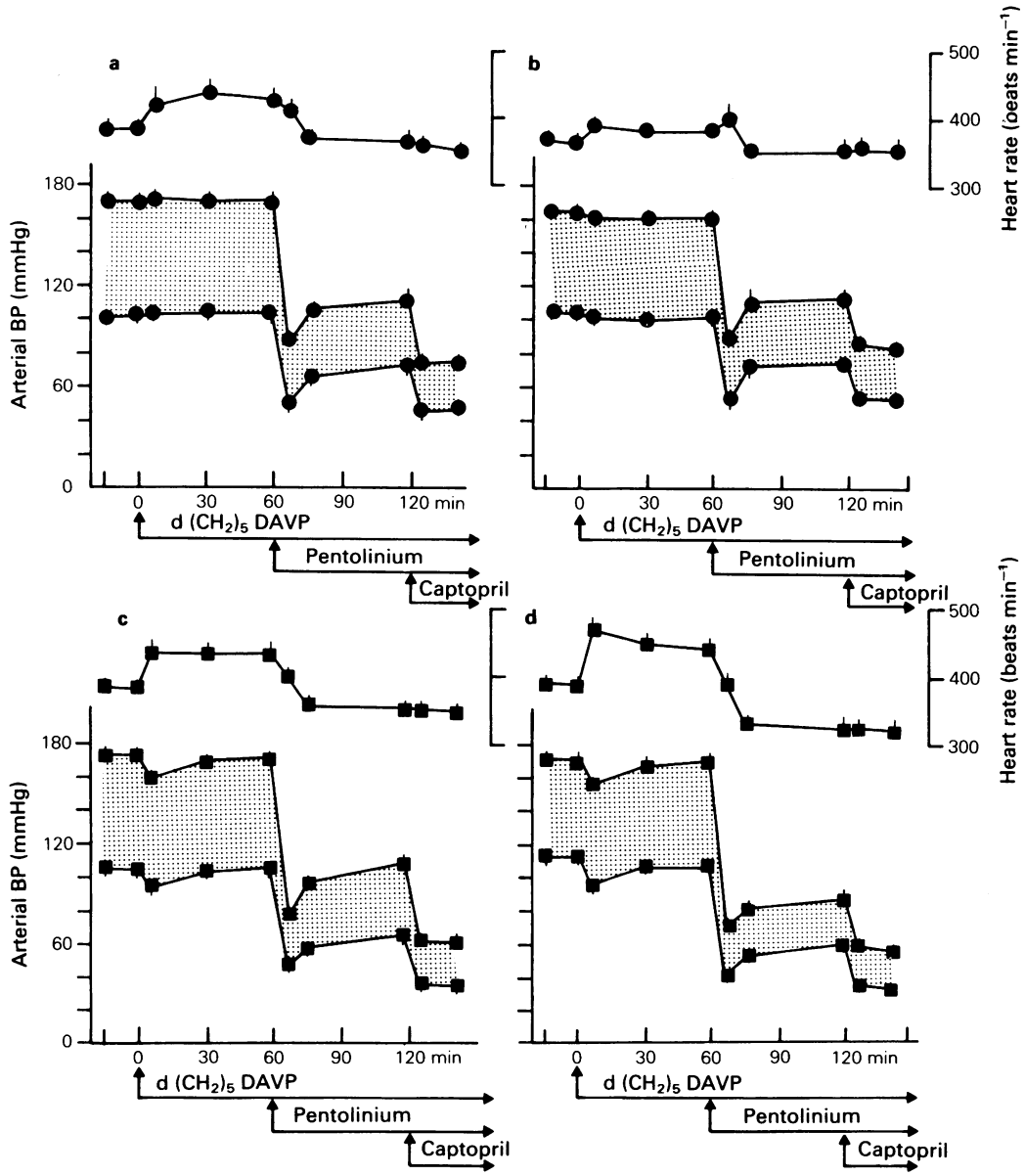


**Figure 1** Arterial blood pressure and heart rate responses to sequential drug administrations in vehicle-injected (a, c) and capsaicin-treated (b, d) rats in the water-replete state (a and b) and following 48 h of water-deprivation (c and d). Values are mean ± s.e.mean; $n = 6$ in each group.

In the capsaicin-treated rats only, resting heart rates were significantly higher ($P < 0.02$) in the water-deprived than in the water-replete state.

The results of the first series of experiments are shown in Figure 1. Administration of $d(CH_2)_5DAVP$ had similar effects in capsaicin-treated and vehicle-injected rats. In the water-replete state $d(CH_2)_5DAVP$ caused no change in arterial blood pressures, but provoked a significant tachycardia ($P < 0.02$ for both groups). In the water-deprived state, $d(CH_2)_5DAVP$ initially caused a significant reduction in systolic (vehicle $P < 0.02$; capsaicin $P < 0.01$) and diastolic (vehicle $P < 0.05$; capsaicin $P < 0.02$) blood pressures, associated with a tachycardia ($P < 0.01$ for both

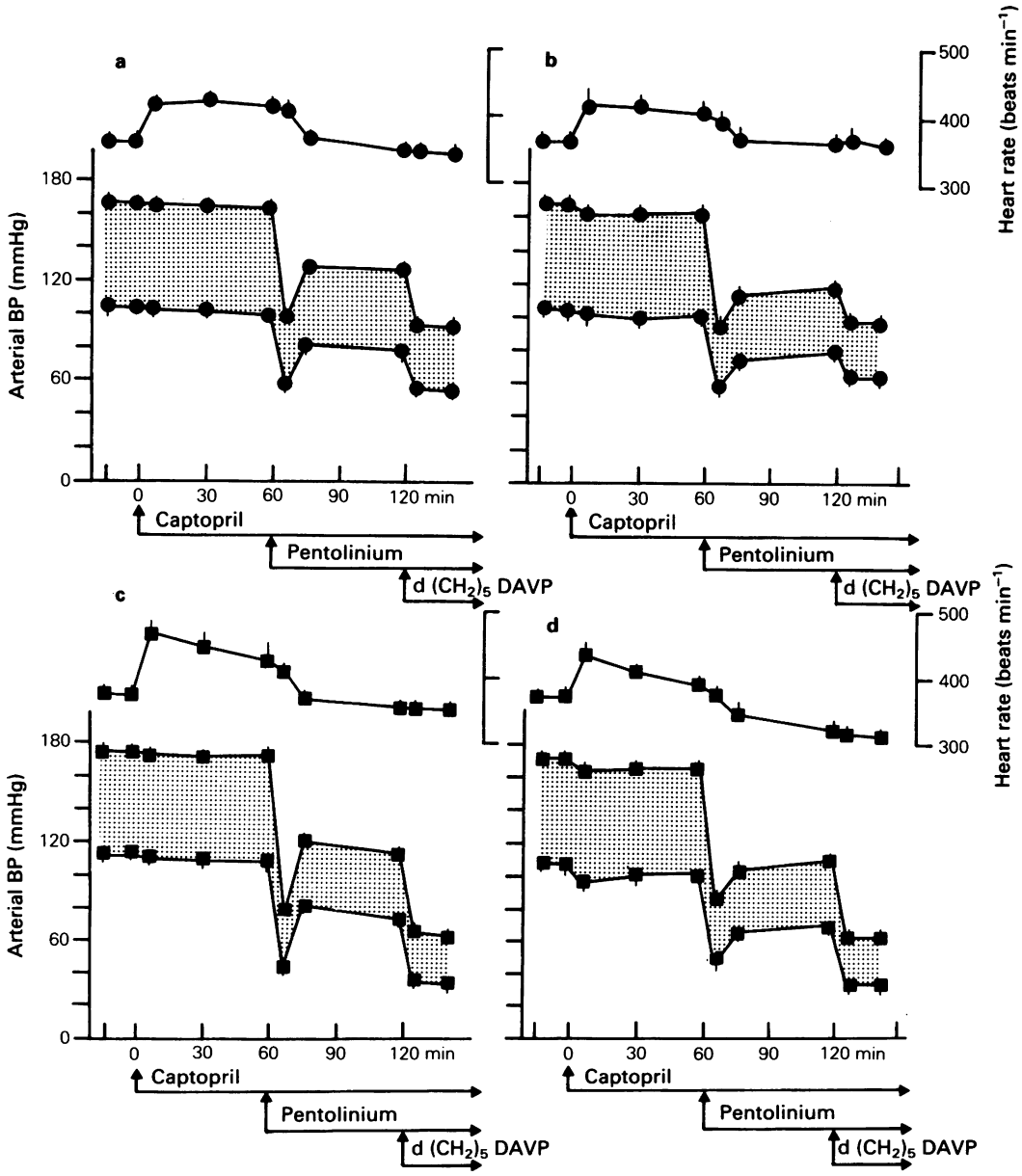


**Figure 2** Arterial blood pressure and heart rate responses to sequential drug administrations in vehicle-injected (a, c) and capsaicin-treated (b, d) rats in the water replete state (a and b) and following 48 h of water deprivation (c and d). Mean values are shown with s.e.mean indicated by vertical lines; $n = 6$ in each group.

groups). During the following 60 min, blood pressures returned to their resting values but heart rates remained high. Subsequent administration of pentolinium caused greater falls in blood pressure in both groups of water-deprived rats than in the water-replete rats ($P<0.05$), but there was no difference between the responses obtained in the vehicle-injected groups compared to those which had received capsaicin. In the presence of $d(CH_2)_5$DAVP and pentolinium, the recovery of blood pressure was similar in the 2 groups of water-replete rats but was impaired in the water-deprived capsaicin-treated rats compared to the vehicle-injected animals ($P<0.001$ for systolic pressure, 60 min after pentolinium). Administration of captopril in the presence of $d(CH_2)_5$DAVP and pentolinium caused a reduction in blood pressure which was similar in both groups of water-replete rats but, the effect was less in the capsaicin-treated, water-deprived rats than in the vehicle-injected, water-deprived group ($P<0.01$ for systolic pressure), with the result that both groups of water-deprived rats had similar blood pressures at the end of the experiment. Blood pressures in the 2 groups of water-deprived rats at the end of the experiment were lower ($P<0.05$) than those measured in the water-replete animals under the same conditions. Furthermore, blood pressures measured at the end of the experiment in the water-deprived animals, were lower ($P<0.05$) than those measured immediately after pentolinium.

The results of the second series of experiments are shown in Figure 2. In vehicle-injected rats, captopril had no significant effect on arterial blood pressure in either the water-replete or the water-deprived state, but it caused a significant tachycardia which was greatest in the water-deprived animals. In capsaicin-treated animals, captopril initially caused a small reduction in blood pressure ($P<0.05$ for systolic in water-replete rats; $P<0.05$ for systolic and diastolic in water-deprived rats 1 min after drug administration), but during the following 60 min blood pressures returned to their resting values. Subsequent administration of pentolinium caused an initial hypotension which was similar in the capsaicin- and vehicle-injected groups; the hypotension was greater in both groups of water-deprived rats than in the water-replete animals, although the difference was significant ($P<0.02$ for systolic and diastolic) only in the vehicle-injected rats. The recovery of blood pressure during the first 10 min of combined captopril and pentolinium administration was less in the capsaicin-treated rats than in the vehicle-injected animals ($P<0.01$ for systolic in water-replete rats; $P<0.02$ for systolic and diastolic in water-deprived rats). However, at the end of the 60 min infusion of pentolinium there was no difference between the blood pressures in any of the 4 groups. $d(CH_2)_5$DAVP given in the presence of captopril and pentolinium caused a reduction in blood pressure in all groups; the change in systolic blood pressure in the water-replete, capsaicin-treated rats was significantly ($P<0.05$) less than in the other groups. At the end of the experiment there were no differences between the blood pressures of the 2 groups of water-replete rats or between the 2 groups of water-deprived rats; both groups of water-deprived rats had lower pressures than the water-replete animals ($P<0.05$ for each case). In both groups of water-deprived rats, blood pressures measured at the end of the experiment were lower ($P<0.05$ in all cases) than those measured immediately after pentolinium.

## Discussion

In the doses we employed there is no evidence that captopril interferes with the cardiovascular actions of vasopressin (Gardiner & Bennett, 1985) or α-adrenoceptor agonists (M. Winn, S.M. Gardiner & T. Bennett, unpublished observations), or that vasopressin antagonists impair the pressor influences of angiotensin II (Crofton *et al.*, 1979) or noradrenaline (Aisenbrey *et al.*, 1981). Furthermore, it is unlikely that the systemic administration of pentolinium interfered with the non-osmotic release of vasopressin (Kuhn, 1974; Knepel & Meyer, 1980; Zerbe *et al.*, 1981). For these reasons we consider that the drugs employed only interfered with the mechanisms we were intending to antagonize. However, pentolinium will inhibit neurally-mediated renin release and impair any influences of angiotensin II and vasopressin expressed through interactions with efferent neural mechanisms. At the end of every experiment, when all three drugs were being given, there was a sustained hypotension, with no indication of other pressor mechanisms being effectively recruited. These observations provide strong evidence that the doses of the drugs used were sufficient to abolish the cardiovascular influences of neural activity, the renin-angiotensin system and vasopressin. Thus our experimental protocols enabled us to assess not only the primary responses to inhibition of either the cardiovascular actions of vasopressin (with $d(CH_2)_5$DAVP) or the renin-angiotensin system (with captopril) but also the recovery of blood pressure attributable either to the direct effects of vasopressin (i.e. in the presence of captopril and pentolinium) or to activation of the renin-angiotensin system independent of neural effects (i.e. in the presence of $d(CH_2)_5$DAVP and pentolinium).

The main aim of the present study was to determine whether or not the apparently normal resting arterial pressures in the water-deprived capsaicin-treated rats were less dependent on vasopressin than in vehicle-injected animals. Our results showed no differences

between capsaicin-treated and vehicle-injected animals in their responses to administration of an antagonist of the vascular effects of vasopressin. In both groups of water-replete animals there was no significant change in blood pressure (although there was a tachycardia) and in both groups of water-deprived animals there was a transient hypotension of similar magnitude, associated with a tachycardia. It is likely that these latter effects were due to similar recruitment of vasopressin-mediated influences in capsaicin-treated and vehicle-injected rats following 48 h water-deprivation, rather than to differential changes in these influences coupled with some obscure abnormality in the interaction between vasopressin and the baroreflex control of blood pressure (Cowley *et al.*, 1983). A normal vasopressin-mediated contribution to the maintenance of blood pressure during water deprivation in the capsaicin-treated rats may appear inconsistent with our earlier findings of impaired vasopressin-mediated effects following acute hypotension in such animals (Bennett & Gardiner, 1984). However, the results of our second experiment show that the impairment which was previously described was not a persistent phenomenon, since 60 min after the onset of pentolinium-induced hypotension (in the presence of captopril) the levels at which blood pressures had stabilized were the same in capsaicin-treated and vehicle-injected animals. In the vehicle-injected rats, blood pressure recovery was maximal within the first 15 min after the pentolinium had been given (in line with the reported changes in plasma vasopressin levels following ganglion blockade in rats; Knepel & Meyer, 1980), whereas in the capsaicin-treated animals, the pattern of blood pressure recovery differed, showing a slower onset and a more gradual rise. However, the response to the vasopressin antagonist at the end of the experiment makes it likely that, in vehicle-injected and capsaicin-treated rats, the recovery of blood pressure was attributable to vasopressin. Since we have shown elsewhere that cardiovascular sensitivity to vasopressin is normal in animals treated neonatally with capsaicin (Bennett & Gardinar, 1985), it appears from the present results that the full extent of the vasopressin-dependent contribution to blood pressure recovery in acute hypotension takes longer to achieve when a large proportion of unmyelinated afferent fibres have been destroyed. We cannot analyse this observation further without information about the rates of release of vasopressin into, and its clearance from, the vascular compartment, and the dynamics of the interaction between vasopressin and $V_1$-receptors.

The results of our second experiment also showed that the impaired vasopressin-mediated blood pressure recovery previously observed in water-deprived, capsaicin-treated rats (Bennett & Gardiner, 1984) occurred also in water-replete animals. This indicates that the previous observations were not attributable to abnormal depletion of pituitary vasopressin levels in water-deprived, capsaicin-treated rats.

The response to the vasopressin antagonist seen in the water-deprived animals was similar to that reported by others in water-deprived Sprague-Dawley rats (Aisenbrey *et al.*, 1981; Andrews & Brenner, 1981). Consistent with a direct contribution from vasopressin to the maintenance of blood pressure in water-deprived Wistar rats, in the present study we found that when pentolinium was given (in the presence of captopril), the pressures did not fall as low as they did at the end of the experiment, when the vasopressin antagonist was also being given, i.e. in the presence of captopril and pentolinium there was a small independent pressor influence of vasopressin. In the experiment where pentolinium was given after the vasopressin antagonist, blood pressure was not reduced to the level seen following additional inhibition of the renin-angiotensin system indicating that angiotensin was also exerting a neurally independent pressor influence. Although it is not possible to state that this influence was present in the water-deprived rats before drugs were administered (rather than being triggered by the hypotension elicited by the vasopressin antagonist), it is noteworthy that following water deprivation, the capsaicin-treated animals showed a fall in blood pressure in response to administration of captopril alone. However, the present results do not permit us to determine if the greater hypotensive effect of captopril in capsaicin-treated rats was due to greater dependence of blood pressure on the renin-angiotensin system or to less effective compensation for inhibition of the renin-angiotensin system, attributable to impairment of the baroreflex release of vasopressin, or to impaired baroreflex control of blood pressure (Bond *et al.*, 1982).

An unexpected finding in the present study was that the water-deprived, capsaicin-treated animals showed some impairment in their neurally-independent, angiotensin-mediated recovery in blood pressure (i.e. following $d(CH_2)_5DAVP$ and pentolinium). One possible explanation for this observation is that, prior to drug administration, angiotensin II receptor occupancy was high (consistent with the hypotensive effect of captopril), resulting in a reduced cardiovascular influence of a further increase in activation of the renin-angiotensin system.

The work was supported by grants from the British Heart Foundation. We are grateful to Professor M. Manning (for $d(CH_2)_5DAVP$), Squibb UK (for captopril) and May & Baker (for pentolinium).

## References


AISENBREY, G.A., HANDELMAN, W.A., ARNOLD, P., MANNING, M. & SCHRIER, R.W. (1981). Vascular effects of arginine vasopressin during fluid deprivation in the rat. *J. clin. Invest.*, **67**, 961–968.

ANDREWS, C.E. JR. & BRENNER, B.M. (1981). Relative contributions of arginine vasopressin and angiotensin II to maintenance of systemic arterial pressure in the anaesthetized water-deprived rat. *Circulation Res.*, **48**, 254–258.

BENNETT, T. & GARDINER, S.M. (1984). Neonatal capsaicin treatment impairs vasopressin-mediated blood pressure recovery following acute hypotension. *Br. J. Pharmac.*, **81**, 341–345.

BENNETT, T. & GARDINER, S.M. (1985). Pressor sensitivity to exogenous vasopressin in conscious adult rats treated neonatally with capsaicin. *Br. J. Pharmac*, **85**, 115–119.

BOND, S.M., CERVERO, F. & McQUEEN, D.S. (1982). Influence of neonatally administered capsaicin on baroreceptor and chemoreceptor reflexes in the adult rat. *Br. J. Pharmac.*, **77**, 517–524.

COWLEY, A.W. JR., QUILLEN, E.W. & SKELTON, M.M. (1983). Role of vasopressin in cardiovascular regulation. *Fedn. Proc.*, **42**, 3170–3176.

CROFTON, J.T., SHARE, L., SHADE, R.E., LEE-KWON, W.J., MANNING, M. & SAWYER, W.H. (1979). The importance of vasopressin in the development and maintenance of DOC-salt hypertension in the rat. *Hypertension*, **1**, 31–38.

GARDINER, S.M., BENNETT, T. & KEMP, P.A. (1980). Systemic arterial hypertension in rats exposed to short-term isolation; intra-arterial systolic and diastolic blood pressures and baroreflex sensitivity. *Med. Biol.*, **58**, 232–239.

GARDINER, S.M. & BENNETT, T. (1985). Interactions between neural mechanisms, the renin-angiotensin system and vasopressin in the maintenance of blood pressure during water deprivation: studies in Long Evans and Brattleboro rats. *Clin. Sci.*, **68**, 647–657.

KNEPEL, W. & MEYER, D.K. (1980). Effect of isoprenaline and trimethidinium on the plasma vasopressin concentration in conscious rats. *Archs Int. Pharmacodyn.*, **245**, 249–261.

KUHN, E.R. (1974). Cholinergic and adrenergic release mechanism for vasopressin in the male rat: A study with injections of neurotransmitters and blocking agents into the third ventricle. *Neuroendocrinology*, **16**, 255–264.

MANNING, M., LAMMED, B., KRUSZYNSKI, M., SETO, J. & SAWYER, W.H. (1982). Design of potent and selective antagonists of the vasopressor responses to arginine vasopressin. *J. med. Chem.*, **25**, 408–414.

NAGY, J.I., HUNT, S.P., IVERSEN, L.L. & EMSON, P.C. (1981). Biochemical and anatomical observations on the degeneration of peptide-containing primary afferent neurons after neonatal capsaicin. *Neuroscience*, **6**, 1923–1934.

RASCHER, W., LANG, R.E., GANTEN, D., MEFFLE, H., TAUBITZ, M., UNGER, T. & GROSS, F. (1983). Vasopressin in deoxycorticosterone acetate hypertension of rats: A haemodynamic analysis. *J. cardiovasc. Pharmac.*, **5**, 418–425.

THOREN, P. (1979). Role of cardiac vagal C-fibres in cardiovascular control. *Rev. Physiol. Biochem. Pharmac.*, **86**, 1–94.

ZERBE, R.L., BAYLIS, P.H. & ROBERTSON, G.L. (1981). Vasopressin function in clinical disorders of water balance. In *The Pituitary* ed. Beardwell, C. & Robertson, G.L. pp. 297–329. London: Butterworths.


*(Received January 22, 1985.*
*Revised March 22, 1985.*
*Accepted April 10, 1985.)*