Br. J. Pharmac. (1985), **85**, 115–119

Pressor sensitivity to exogenous vasopressin in conscious, adult rats treated neonatally with capsaicin

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1 Pressor responses to exogenous arginine vasopressin were assessed in adult rats that had been treated neonatally with capsaicin or its vehicle. Measurements were made under control conditions, after inhibition of baroreflexes (with pentolinium), and after inhibition of baroreflexes (with pentolinium) and the production of angiotensin II (with captopril).

2 Resting arterial blood pressures and pressor sensitivities to exogenous arginine vasopressin were similar in capsaicin-treated and vehicle-injected rats.

3 Sixty minutes after the administration of pentolinium, systolic and diastolic blood pressures were reduced in both groups of rats and the pressor responses to arginine vasopressin were similarly and significantly enhanced.

4 In both groups of rats 60 min after administration of pentolinium and captopril, systolic and diastolic blood pressures were lower than in the presence of pentolinium alone, but pressor responses were not different from those seen in control conditions.

5 The possibility that the present results are explicable in terms of baroreflexes, the renin-angiotensin system and endogenous vasopressin interacting to influence the pressor sensitivity to exogenous vasopressin is discussed. From the present findings, it seems that our previous observation of impaired, vasopressin-mediated blood pressure recovery following acute hypotension in capsaicin-treated rats cannot be attributed to a reduced pressor sensitivity to the hormone.

Introduction

We have recently demonstrated impaired vasopressin-mediated blood pressure recovery following acute hypotension in water-deprived, adult rats that had been treated neonatally with a dose of capsaicin which selectively destroys unmyelinated afferent nerve fibres (Bennett & Gardiner, 1984). This we interpreted as being due to capsaicin-induced diminution of the reflex release of vasopressin, consistent with the suggestion that unmyelinated nerve fibres are involved in the afferent pathways mediating the non-osmotic release of vasopressin (Thoren, 1979); but the possibility remained that the capsaicin-treated animals had a normal reflex release of vasopressin and were less sensitive to the cardiovascular actions of the hormone. We have now tested the responsiveness of capsaicin-treated animals to the pressor effects of exogenous vasopressin. However, since the pressor responses to vasopressin are undoubtedly influenced by baroreceptor-mediated reflex activity (Cowley *et al.*, 1983), and since there is evidence that, in the anaesthetized state, capsaicin-treated rats show impaired baroreflex control of blood pressure (Bond *et al.*, 1982) but not heart rate (Lorez *et al.*, 1983), it was necessary to assess the baroreceptor effect on the pressor responses to vasopressin in our conscious rats. This was done, firstly, by assessing the baroreflex control of pulse-interval (Smyth *et al.*, 1969) and, secondly, by comparing pressor responses to vasopressin in the absence and presence of a ganglion blocker.

Following ganglion blockade in rats, the circulating levels of angiotensin II and vasopressin increase (Knepel & Meyer, 1980). In the light of recent evidence suggesting a positive influence of the former on the sensitivity to the latter (Ishikawa *et al.*, 1984), we performed a further experiment in which pressor responses to vasopressin were measured in the presence of a ganglion blocker and a converting enzyme inhibitor.

Methods

Wistar rats were anaesthetized with halothane (in oxygen) on day 2 after birth and injected subcutan-

eously with either vehicle (10% ethanol, 10% Tween 80 in 0.9% w/v NaCl solution) or capsaicin (50 mg kg^{-1}) in a volume of 10 μl. Forty weeks later rats were anaesthetized with sodium methohexitone (60 mg kg^{-1}, intraperitoneally), and catheters, filled with heparinized saline (12.5 units ml^{-1}) were implanted in the jugular vein (2 or 3 catheters) for drug administration, and in the abdominal aorta (1 catheter) via the caudal artery for blood pressure measurements. Access to the jugular vein and caudal artery required superficial surgery only, during which 0.5% lignocaine (Astra Pharmaceuticals) was applied liberally to blood vessels and the surrounding tissues. Heart rate was derived from the blood pressure recording. Details of the catheter design and recording system, which permits accurate measurement of systolic and diastolic blood pressures, have been described elsewhere (Gardiner *et al.*, 1980).

The experiment began at least 5 h after surgery, when the animals were fully conscious. At this time the animals did not appear to be in pain and were moving freely, drinking, passing urine and, occasionally, eating. Objective evidence that the animals were in a steady state at this time comes from the finding that there are no significant changes in baroreflex sensitivities between 5 and 7 h after surgery (Gardiner *et al.*, 1980). Furthermore, it has been shown that plasma vasopressin concentrations are normal in conscious, freely-moving rats 4 h after surgery more extensive than that involved in the present experiments (Rascher *et al.*, 1983). Drug administrations were started after a 30 min period of baseline recordings. In 2 groups (vehicle, $n = 6$; capsaicin, $n = 6$) arterial blood pressure was increased by infusion of methoxamine (0.4 mg ml^{-1}; 0.2 ml min^{-1}) and decreased by injection of a bolus of sodium nitroprusside (100 μg ml^{-1}; 0.1 ml); the slopes of the lines relating systolic blood pressure to the pulse-interval of the succeeding beat were obtained by linear regression analysis and used as an index of baroreflex sensitivity (Smyth *et al.*, 1969). Thirty minutes after the last baroreflex test, the pressor responses to arginine vasopressin (6.25, 12.5 and 25 ng kg^{-1}) were measured.

In another 2 groups (vehicle, $n = 6$; capsaicin, $n = 7$) pentolinium (5 mg kg^{-1} bolus; 5 mg kg^{-1} h^{-1} infusion) was administered for 60 min; whilst the infusion was continued, pressor responses to arginine vasopressin (as above) were measured. In a further two groups (vehicle, $n = 6$; capsaicin, $n = 6$) captopril (2 mg kg^{-1} bolus; 1 mg kg^{-1} h^{-1} infusion) plus pentolinium (as above) were given for 60 min; whilst the infusions were continued, the pressor responses to arginine vasopressin (as above) were measured. The dose of captopril used was sufficient to abolish the pressor effects due to 125 ng of angiotensin I and did not, itself, affect vasopressin sensitivity (Gardiner & Bennett, 1985). The dose of pentolinium used abolished the reflex bradycardia resulting from a 50 mmHg rise in systolic blood pressure evoked by methoxamine. Furthermore, in the presence of this dose of pentolinium there was no evidence of residual sympatho-adrenal activity following inhibition of the renin-angiotensin system and the vascular actions of vasopressin (Gardiner & Bennett, 1985).

Values are expressed as the mean ± 1 s.e.mean; n is the number of animals. Results were analysed for statistical significance by the Mann Whitney U-test.

The following drugs were used: methoxamine hydrochloride (Burroughs Wellcome), sodium nitroprusside (Roche), arginine vasopressin (Cambridge Biochemicals), pentolinium tartrate (May & Baker) and captopril (Squibb).

Results

Resting arterial blood pressures (systolic/diastolic) and heart rates in capsaicin-treated rats (165 ± 2/104 ± 1 mmHg; 350 ± 5 beats min^{-1}; $n = 19$) were not different from those in vehicle-injected animals (168 ± 2/104 ± 2 mmHg; 348 ± 5 beats min^{-1}; $n = 18$).

The slopes of the lines relating systolic blood pressure to pulse-interval during methoxamine infusion were not different in the 2 groups (capsaicin = 1.31 ± 0.10 ms $mmHg^{-1}$; vehicle = 1.23 ± 0.09 ms $mmHg^{-1}$). Likewise, there were no differences in the pulse-interval responses to a depressor stimulus between capsaicin-treated rats (0.97 ± 0.11 ms mm Hg^{-1}) and vehicle-injected animals (1.07 ± 0.08 ms $mmHg^{-1}$).

Pressor responses to arginine vasopressin were similar in the two groups when baroreflexes were intact (Figure 1).

After pentolinium, blood pressures fell significantly ($P < 0.001$), but 60 min later had stabilized at similar levels in the 2 groups (capsaicin = 124 ± 5/76 ± 2 mmHg; vehicle = 129 ± 7/86 ± 5 mmHg). Systolic blood pressure responses to arginine vasopressin were significantly augmented in both groups receiving pentolinium compared to controls (Figure 1; $P < 0.001$ at each dose level) but there were no differences between the responses obtained in capsaicin-treated and vehicle-injected rats. A similar picture was obtained for the diastolic blood pressure responses to arginine vasopressin, although in the vehicle-treated animals the response to the lowest dose was not significantly different from that seen in the control group (Figure 1).

On administration of pentolinium and captopril, blood pressures initially fell to similar levels in the 2 groups (vehicle = 84 ± 6/49 ± 4; capsaicin = 77 ± 5/42 ± 3 mmHg). During the infusion of the drugs, blood pressure recovery was less effective in the capsaicin-treated group than in the controls (15 min after administration, vehicle = 98 ± 6/62 ± 6; cap-

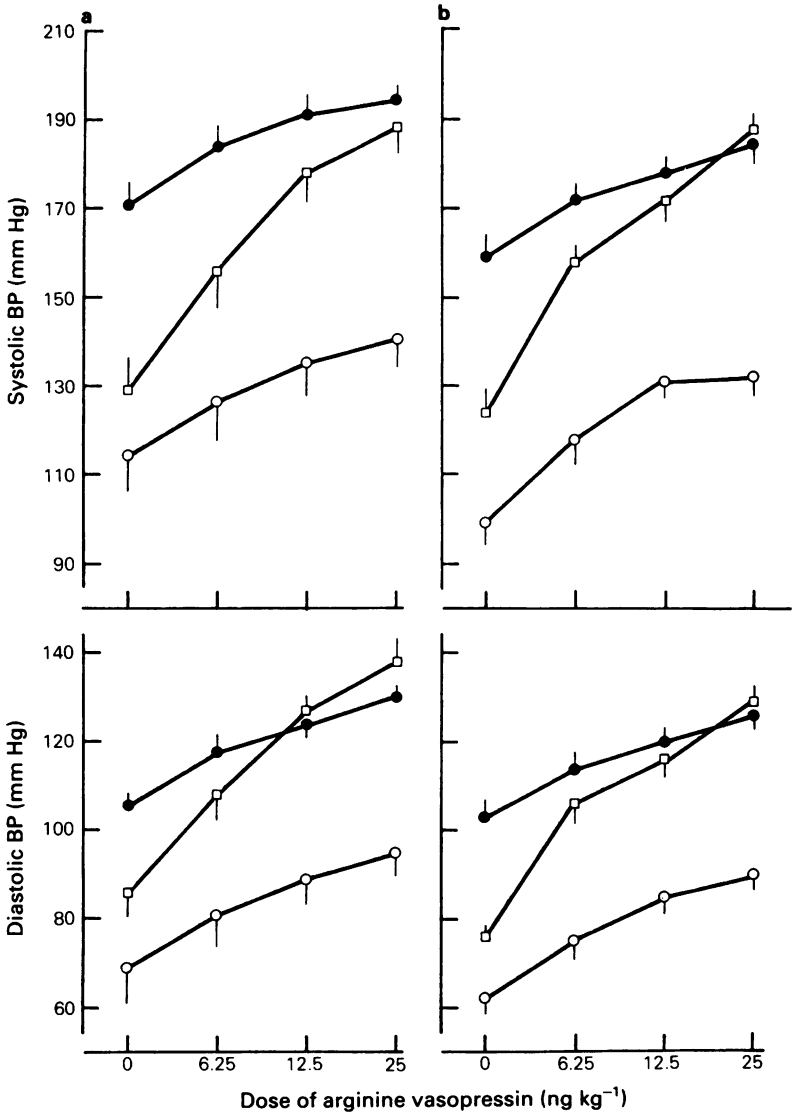

Figure 1 Pressor responses to arginine vasopressin in vehicle (a)- and capsaicin (b)-treated rats under control conditions (●) and 60 min after treatment with pentolinium (□) or pentolinium plus captopril (○). Each point shows the mean and vertical lines indicate s.e.means.

saicin = 83 ± 3/49 ± 2 mmHg: $P < 0.001$; 60 min after administration, vehicle = 114 ± 8/69 ± 7; capsaicin = 99 ± 1/62 ± 2 mmHg: $P < 0.05$ for systolic blood pressure). During administration of pentolinium and captopril, blood pressures stabilized at levels that were lower than in the presence of pentolinium alone, but the difference was significant ($P < 0.001$) only in the capsaicin-injected animals. In both groups, pressor responses to vasopressin were markedly less than those seen during administration of pentolinium alone and were not significantly different from the responses seen in animals with intact baroreflexes (Figure 1).

Discussion

Although we did not make direct assessments of the extent of capsaicin-induced lesions in the present study, the protocol we followed has been found to cause a selective and permanent destruction of about

90% of the peripheral, unmyelinated afferent fibres (Nagy *et al.*, 1981). Furthermore, the impaired blood pressure recovery in capsaicin-treated rats which we observed following administration of pentolinium and captopril is consistent with our previous findings (Bennett & Gardiner, 1984) and assured us that the capsaicin-treated animals used in this study had been affected to a similar extent as those used previously.

The present results obtained in conscious animals are similar to those obtained under anaesthesia (Bond *et al.*, 1982; Lorez *et al.*, 1983) which showed that adult rats, treated neonatally with capsaicin, had normal, resting arterial blood pressures. Thus, the possibility that capsaicin-treated rats appear normotensive under anaesthesia due to an effect of the anaesthetic on blood pressure (Bond *et al.*, 1982) can be discounted. However, it is possible that baroreceptor deafferentation following neonatal treatment with capsaicin produces a greater lability of blood pressure, as seen following carotid sinus denervation (Norman *et al.*, 1981). We are now investigating the factors contributing to the apparently normal blood pressures in rats treated neonatally with capsaicin.

We extended the earlier work of Lorez *et al.* (1983) on anaesthetized rats, and showed that, in the conscious state, there were no differences between the pulse-interval responses to pressor or depressor stimuli in capsaicin-treated rats and those in vehicle-injected rats. We also found that both groups of animals had similar pressor sensitivities to exogenous arginine vasopressin. However, it was theoretically possible that this similarity resulted from a decreased vascular sensitivity to vasopressin coupled with a decreased buffering of the vasoconstrictor effects of vasopressin by reflex withdrawal of vasomotor tone in the capsaicin-treated animals. Such an abnormality of baroreflex function would not necessarily have been detected by assessing heart rate changes only (Zanchetti, 1979). In order to investigate this possibility we also assessed vasopressin sensitivity in animals rendered areflexic with pentolinium and found that the responsiveness of capsaicin-treated and control animals was the same, with both groups showing a marked enhancement in sensitivity to vasopressin under these conditions. While the latter observation is not new (Matsuguchi & Schmid, 1982), the findings from our final experiment indicate that this phenomenon might involve more factors than have been considered previously; as, in the presence of pentolinium and captopril (in a dose that had no direct effect on sensitivity to vasopressin; Gardiner & Bennett, 1985) the pressor sensitivity to vasopressin was the same as in the absence of any drugs. Since the basal blood pressures were lower in the presence of captopril and pentolinium than in the presence of pentolinium alone, we would have expected a greater increase in pressor sensitivity to vasopressin in the former than in the latter conditions (Hoffman, 1980). We suggest an explanation for these observations might lie with differential activation of endogenous pressor systems under the various experimental conditions studied. Following ganglion blockade in rats the renin-angiotensin system is stimulated (Knepel & Meyer, 1980) and blood pressure recovery is dependent on this more than on endogenous vasopressin (Bennet & Gardiner, 1983). While there is no evidence that this is due to ganglion blockade impairing the non-osmotic release of vasopressin (Kuhn, 1974; Knepel & Meyer, 1980; Zerbe *et al.*, 1981), it is possible that antagonism of nicotinic transmission enhances vasopressin release by inhibiting the baroreceptor-mediated suppression of vasopressin release (Bisset & Chowdrey, 1984). However, hypovolaemia elicited by blood withdrawal or administration of a ganglion-blocker has been shown to produce similar elevations in plasma vasopressin levels (Zerbe *et al.*, 1981). Therefore, the enhanced sensitivity to exogenous vasopressin in the presence of pentolinium could have been due to abolition of baroreflexes (Cowley *et al.*, 1983) coupled with a peripheral synergism between angiotensin II and exogenous vasopressin (Ishikawa *et al.*, 1984) under conditions in which vasopressin receptors were not exposed to excessive levels of the endogenous neurohormone. Following concurrent ganglion blockade and inhibition of the renin-angiotensin system, blood pressure becomes dependent on vasopressin (Bennett & Gardiner, 1983). Thus, under these conditions, sensitivity to exogenous vasopressin could have been decreased directly due to receptor occupancy by the endogenous neurohormone, and indirectly, due to the dimunition in the synergistic effect of angiotensin II. Both these factors would act to offset the enhancing effect of baroreflex inhibition on sensitivity to vasopressin.

Of course, the above explanations for our results are likely to be simplistic since they do not consider all the possible consequences of the use of captopril as a means of inhibiting the renin-angiotensin system (Zusman, 1984). However, to return to our original aims, in no circumstances did we find that rats treated neonatally with capsaicin were less sensitive to exogenous vasopressin than were vehicle-injected animals. These results corroborate our suggestion that abnormality of vasopressin-mediated recovery in blood pressure following treatment with captopril and pentolinium is due to impairment of reflex release of vasopressin (Bennett & Gardiner, 1984).

This work was supported by grants from the British Heart Foundation. We are grateful to Squibb UK (for captopril) and May & Baker (for pentolinium).

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(Received August 27, 1984.
Revised December 24, 1984.
Accepted January 17, 1985.)