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Neonatal capsaicin treatment impairs vasopressin-mediated blood pressure recovery following acute hypotension

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1 Rats were treated with a single injection of either capsaicin (50 mg kg^{-1} s.c.) or vehicle on day 2 after birth. When the animals were adult, they were challenged with osmotic (water deprivation) and haemodynamic (acute hypotension) stimuli that normally evoke vasopressin release.

2 Capsaicin-treated and vehicle-injected rats showed similar body weight losses and plasma osmolalities following 48 h of water deprivation. Thus it appears that neonatal treatment with capsaicin does not impair the antidiuretic response to plasma hyperosmolality.

3 Following acute ganglion blockade in the presence of angiotensin converting enzyme inhibition, there was some recovery of blood pressure in the vehicle-injected rats, but recovery was significantly ($P < 0.001$) less in the capsaicin-treated animals. The recovery may be attributed to vasopressin since it was abolished by an antagonist selective for the pressor action of the peptide ($d(CH_2)_5DAVP$).

4 These results suggest that neonatal treatment with capsaicin impairs vasopressin-mediated recovery of blood pressure following acute hypotension. The possible involvement of baro- or chemoreceptor afferents is discussed.

Introduction

Studies directed at determining the role of capsaicin-sensitive afferents in cardiovascular reflexes have produced largely negative results (Furness, *et al.*, 1982; Lorez *et al.*, 1983). However, those investigations examined the baroreflex control of heart rate which involves mainly myelinated afferent fibres, whereas the destructive effects of capsaicin are mostly confined to unmyelinated fibres (Nagy *et al.*, 1981). Since there is some suggestion that unmyelinated afferents may be involved in the release of vasopressin (Thoren, 1979), we have examined the responses of rats treated neonatally with capsaicin to water deprivation and acute hypotension—osmotic and haemodynamic stimuli that normally evoke vasopressin release.

Present evidence indicates that the renin-angiotensin system, sympathetic nervous system and vasopressin may all interact and contribute to the maintenance of arterial blood pressure in normal (Gavras *et al.*, 1982) and water-deprived states (Andrews & Brenner, 1981). We therefore used an angiotensin-converting enzyme inhibitor (captopril) and a ganglion blocker (pentolinium) to achieve an acute hypotension in which a possible influence of vasopressin on blood pressure could be discerned. The involvement of vasopressin under these conditions was confirmed by administration of $d(CH_2)_5DAVP$, a selective antagonist of the pressor action of vasopressin.

For futher comparisons, a group of rats congenitally unable to synthesize vasopressin (Brattleboro strain) were deprived of water and the effects of captopril and pentolinium on blood pressures were measured.

Methods

Three groups of Wistar rats were used: Group (a) normally hydrated controls (5 males, 240–280 g); Group (b) rats injected on day 2 after birth with vehicle (10% ethanol, 10% Tween 80 in 0.9% NaCl; 10 µl subcutaneously) and deprived of drinking water for the 48 h before the experiment (3 males and 4 females, 180–285 g); Group (c) rats injected on day 2 after birth with capsaicin (50 mg kg^{-1} in a volume of

10 μl; Nagy *et al.*, 1981) and deprived of drinking water for the 48 h before the experiment (3 males and 4 females, 176–280 g). Neonatal rats were anaesthetized with halothane before injection.

In addition, one group of homozygous Brattleboro rats (6 males, 260–300 g) was used. These animals were deprived of water for 8 h before the experiment.

On the day of the experiment, rats were anaesthetized (sodium methohexitone, 60 mg kg^{-1} i.p.) and catheters filled with heparinized saline (12.5 u ml^{-1}) were implanted (3 in the jugular vein for drug administrations and 1 in the abdominal aorta for blood pressure recording). The experiment was carried out 5 h later when the animals were fully conscious. Although it is likely that some methohexitone may still be present at this time, we have no evidence that the anaesthetic is affecting our results (Gardiner *et al.*, 1980).

Details of the catheter design and recording system have been described elsewhere (Gardiner *et al.*, 1980). Baseline recordings were made for 20 min, after which captopril was given (2 mg kg^{-1} bolus; 1 mg $kg^{-1}h^{-1}$infusion) followed 1 h later by pentolinium (5 mg kg^{-1} bolus; 5 mg $kg^{-1}h^{-1}$infusion) and finally a vasopressin antagonist $d(CH_2)_5$DAVP (1-β-mercapto,-β, β-cyclopentamethylenepropionic acid, 8-D-arginine vasopressin; 10 μg kg^{-1}) 10–12 min after pentolinium. $d(CH_2)_5$DAVP is highly selective for the pressor action of vasopressin and was supplied by Professor M. Manning from the Medical College of Ohio. The doses of captopril and $d(CH_2)_5$DAVP were sufficient to abolish the pressor effects of 125 ng angiotensin I and of 5 mu of vasopressin respectively for the duration of the experiment. At the end of the experiment blood was taken from the dehydrated animals for the measurement of plasma osmolality. (In this laboratory the plasma osmolality of normally hydrated Wistar rats is between 295 and 305 mosm kg^{-1}).

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

Results

Since there was no difference between any of the responses obtained in males and females, the results have been pooled. Water deprivation for 48 h caused similar losses in body weight and resulted in indistinguishable plasma osmolalities in Wistar rats treated neonatally with vehicle or capsaicin (−27 ± 1 g and −25 ± 2 g; 321 ± 1 mosm kg^{-1} and 322 ± 1 mosm kg^{-1} respectively). Thus it would appear that the capsaicin treatment had not impaired the ability to cope with water deprivation, i.e. to mount an antidiuretic response to increased plasma osmolality.

In comparison, the body weight loss experienced by the Brattleboro rats after only 8 h of water deprivation was 35 ± 3 g and the plasma osmolality was 339 ± 5 mosm kg^{-1}.

There were no differences between the resting arterial blood pressures in the three groups of Wistar rats (Figure 1). In control, normally hydrated Wistar rats (Figure 1a) captopril did not affect blood pressure but subsequent administration of pentolinium caused a prompt hypotension. During the ensuing 10 min there was a progressive recovery in blood pressure which we attribute to vasopressin for two reasons. Firstly, in the Wistar rats it was abolished by the vasopressin antagonist (Figure 1a) and secondly, in the Brattleboro rats blood pressures fell to 36 ± 1:17 ± 1 mmHg (systolic:diastolic) immediately after the pentolinium adminstration and there was no subsequent recovery (36 ± 1:18 ± 1 mmHg 10 min later).

In vehicle-injected, dehydrated, Wistar rats (Figure 1b) there was a slight fall in blood pressure following captopril ($P < 0.05$ for systolic and diastolic 5 min after administration). After pentolinium, blood pressures initally fell to a level that was significantly ($P < 0.01$) lower than in the hydrated animals but during the ensuing 10 min the recovery was greater with the result that the blood pressures in the hydrated and dehydrated groups were not significantly different immediately before the vasopressin antagonist was given. Following $d(CH_2)_5$DAVP, blood pressures fell to and remained at a significantly ($P < 0.001$) lower level in the dehydrated group, presumably due to the animals' state of volume depletion.

The capsaicin-treated, dehydrated Wistar rats (Figure 1c) showed a hypotension in response to captopril ($P < 0.01$ for systolic and diastolic 5 min after administration) which was greater (but not significantly so) than the response in the vehicle-injected, dehydrated group. Following pentolinium, blood pressures initially fell to a similar level in the 2 groups of dehydrated rats but the capsaicin-treated group showed a significantly ($P < 0.001$) depressed recovery. After $d(CH_2)_5$DAVP the response was smaller ($P < 0.001$) in the capsaicin-treated Wistar rats with the result that their blood pressures at the end of the experiment were not different from the vehicle-injected Wistar rats at that time.

It is interesting to note that in the hydrated Wistar rats following pentolinium (in the presence of captopril) the level to which blood pressures initially fell was the same as that reached with subsequent addition of $d(CH_2)_5$DAVP; however, in both groups of dehydrated Wistar rats, blood pressures were lower

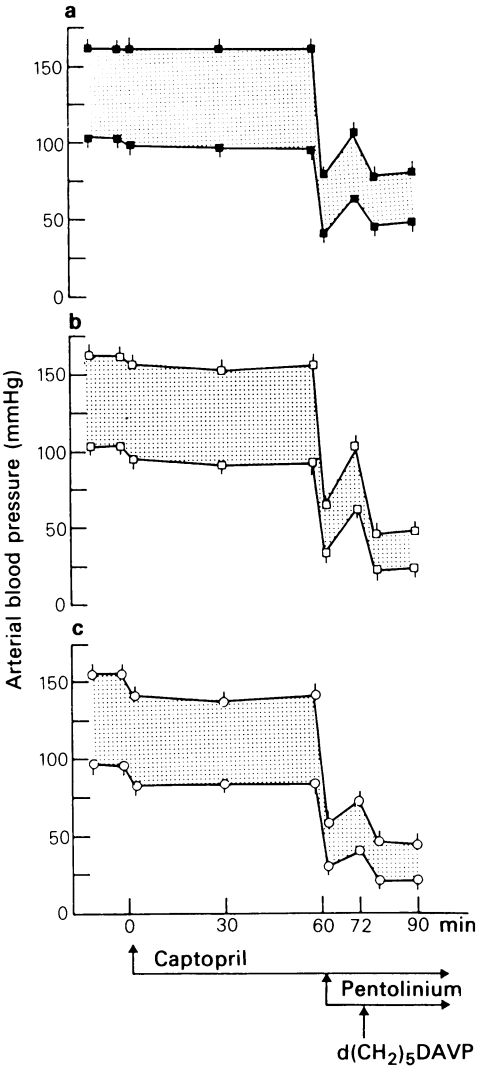

Figure 1 The effects of sequential treatment with captopril (2 mg kg^{-1}; 1 mg kg^{-1}h^{-1} infusion), pentolinium (5 mg kg^{-1}; 5 mg kg^{-1}h^{-1} infusion) and d(CH$_2$)$_5$DAVP (10 μg kg^{-1}) on systolic and diastolic arterial blood pressures (mean ± with s.e.mean) in control hydrated rats (a; $n = 5$) and 48 h water-deprived rats treated neonatally with vehicle (b; $n = 7$) or capsaicin (c; $n = 7$). There was no difference between the resting arterial pressures in the 3 groups. Captopril caused no change in blood pressures in hydrated rats but reduced blood pressures in both groups of dehydrated animals. Following pentolinium the capsaicin-treated rats showed the least tendency to recover. The response to d(CH$_2$)$_5$DAVP was significantly ($p < 0.001$) less in the capsaicin-treated rats than in the vehicle-injected group. At the end of the experiment the arterial pressures of the two dehydrated groups was less than in the hydrated group, presumably due to their state of volume depletion.

in the presence of all 3 drugs than following pentolinium plus captopril. This might be taken as evidence for the involvement of vasopressin in the maintenance of blood pressure following captopril treatment in the dehydrated, but not in the hydrated, state.

Discussion

In the first part of this study we assessed the ability of rats treated neonatally with capsaicin to cope with dehydration. Measurements of body weight losses and plasma osmolalities revealed no difference between capsicin-treated Wistar rats and vehicle-injected animals following 48 h of water deprivation. As a comparison, rats congenitally unable to synthesize vasopressin (Brattleboro strain) showed an equivalent loss in body weight after only 8 h of water deprivation. Thus it appears that rats treated with capsaicin neonatally have a normal antidiuretic response to plasma hyperosmolality.

In the second part of the study we assessed the ability of dehydrated, capsaicin-treated, Wistar rats to cope with acute hypotension by measuring the effects on blood pressure of sequentially inhibiting the renin-angiotensin system, the sympathetic nervous system, and finally the action of vasopressin on the vasculature. Captopril had no effect on blood pressure in the hydrated control animals. In dehydrated, vehicle-treated or capsaicin-treated Wistar rats, captopril caused a small (but significant) reduction in blood pressure, indicating some involvement of the renin-angiotensin system in the maintenance of blood pressure in rats deprived of water for 48 h. It is possible that the change seen with captopril was not larger because activation of the renin-angiotensin system by hypovolaemia due to dehydration may be offset by the accompanying hypernatraemia (Shade *et al.*, 1972) and elevated levels of plasma vasopressin (Bunag *et al.*, 1966), both of which act to inhibit renin release.

Following ganglion blockade (in the presence of captopril) the level to which blood pressures initially fell was similar in both groups of dehydrated Wistar rats and lower than in the hydrated animals; this presumably reflects the reduced plasma volumes of the former. However, in the vehicle-injected Wistar rats the recovery of blood pressure that followed was greater than in the control, hydrated animals. We believe that this recovery of blood pressure was attributable to vasopressin being released in response to the hypotension, firstly because it was abolished by a specific vasopressin antagonist (d(CH$_2$)$_5$DAVP; Manning *et al.*, 1982) and secondly because it did not occur in the Brattleboro rats which lack vasopressin. The greater vasopressin-mediated effect in the de-

hydrated, vehicle-injected animals compared to the hydrated controls could be explained by the observation that vasopressin release in response to hypotension is augmented when plasma osmolality is increased (Dunn *et al.*, 1973).

The recovery of blood pressure following treatment with captopril and pentolinium, and the subsequent response to the vasopressin antagonist were markedly reduced in the capsaicin-treated Wistar rats compared to vehicle-injected animals. These observations indicate that vasopressin release in response to hypotension was impaired in Wistar rats treated neonatally with capsaicin. Impaired vasopressin release in response to hypotension in the presence of an apparently normal response to water deprivation is consistent with some clinical observations (Zerbe *et al.*, 1983) and might be taken as support for the belief that the osmoreceptor and baroreceptor mechanisms involved in vasopressin release are distinct systems (Schrier & Bichet, 1981). However, in the present experiments, it is also possible that the baroreceptor control of vasopressin release appeared abnormal whereas osmoreceptor control did not because the antidiuretic effects of vasopressin are exerted at concentrations much lower than those that have overt cardiovascular effects. The present results are also open to another interpretation; the degree of hypotension caused by ganglion blockade may have elicited vasopressin release by activating chemoreceptors (Share & Levy, 1966; Raff *et al.*, 1983). Thus a reduction in peripheral chemosensitivity in capsaicin-treated rats (Bond *et al.*, 1982) could also explain the decreased vasopressin response to acute hypotension in these animals.

It is interesting to note that our results provide a possible explanation for the observation that capsaicin-treated rats show a reduced pressor response to bilateral carotid occlusion (Bond *et al.*, 1982) since the latter manoeuvre normally stimulates vasopressin release (Share, 1976), which presumably contributes to the pressor response.

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