The effects of infusions of arginine vasopressin or 1-deamino-8-D-arginine vasopressin on common carotid vascular resistance in conscious, Long Evans rats

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- 1 Intravenous infusions of arginine vasopressin or 1-deamino-8-D-arginine vasopressin (DDAVP) were given to conscious, Long Evans rats chronically instrumented with bilateral, common carotid, pulsed Doppler probes and intravascular catheters.
- 2 During infusion of vasopressin at $0.3 \text{ nmol min}^{-1}$ there was an increase in common carotid vascular resistance with no change in mean blood pressure or heart rate. Following infusion there was a common carotid vasodilatation.
- 3 During infusion of vasopressin at $3.0 \text{ nmol min}^{-1}$ there were increases in mean arterial blood pressure and in common carotid vascular resistances, accompanied by bilateral reductions in flow and in heart rate. Administration of (+)-(CH₂)₅Tyr(Et)DAVP (a V₁-receptor antagonist), during the continued infusion of vasopressin, reversed the effects of the latter on mean blood pressure and heart rate; under these conditions there were increases in common carotid blood flows above baseline, in company with bilateral vasodilatations. The latter effects persisted after cessation of vasopressin infusion.
- 4 Infusions of DDAVP were without significant effects on any measured cardiovascular variable.
- 5 The results do not provide straightforward support for the claim that vasopressin acts to promote cerebral perfusion, at least when V₁-receptor effects are unopposed. Furthermore, it seems likely that the vasodilator influence of vasopressin on the common carotid vascular bed is not due to stimulation of V₂-receptors.

Introduction

In vitro observations on the responses of canine basilar artery preparations to vasopressin have shown these vessels can respond with an endothelium-dependent relaxation (Katusic *et al.*, 1984) and it has been suggested that vasopressin-induced vasoconstriction elsewhere could thus favour cerebral perfusion during haemorrhage or septic shock (Katusic *et al.*, 1984). However, subsequent studies with common carotid artery preparations from rats showed these vessels responded to vasopressin with an endothelium-independent vasoconstriction (Katusic & Krstic, 1987), although this effect was less marked than those seen in the aorta and renal artery. Katusic & Krstic (1987) suggested, therefore, that their observations were consistent with the hypothesis that elevated circulating levels of vasopressin would favour perfusion of the cerebral

circulation. Since there have been no studies of the effects of vasopressin on common carotid vascular resistances in conscious rats we addressed this question in the present work and also investigated the effects of the V₂-receptor agonist, 1-deamino-8-D-arginine vasopressin (DDAVP). In addition, we assessed the effects of V₁-receptor antagonism on the carotid vascular responses to vasopressin.

Methods

Male, Long Evans rats (350–450 g) were anaesthetized (sodium methohexitone, Eli Lilly, 60 mg kg^{-1} i.p., supplemented as required to maintain abolition of the toe-pinch reflex) and had pulsed Doppler flow probes (Haywood *et al.*, 1981) implanted around left and right common carotid arteries. After the vessels had been exposed through a mid-line incision, they were gently separated from

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Table 1 Baseline heart rate, blood pressure, Doppler shift and carotid vascular resistances in Long Evans rats

Heart rate (beats min ⁻¹)	Mean blood pressure (mmHg)	Doppler shift (kHz)		Vascular resistance (mmHg kHz ⁻¹)	
		Left carotid	Right carotid	Left carotid	Right carotid
351 ± 12	99 ± 2	3.1 ± 0.4	2.8 ± 0.4	34 ± 4	41 ± 7

Values are mean ± s.e.mean (*n* = 8).

the accompanying nerve trunks, so that suturing the probes (Crystal Biotech, Holliston, U.S.A.) in position did not damage the nerve trunks, as assessed by intact bradycardic or tachycardic responses respectively to hypertensive and hypotensive stimuli and the absence of ptosis. The wires from the probes were tunnelled subcutaneously and were exteriorized at the back of the neck where they were soldered into a microconnector held in a harness worn by the rat (Microtech, Inc., Boothwyn, U.S.A.). Animals were allowed to recover for at least 7 days, after which time those with acceptable signals from both probes (signal:noise ratio, >20:1) were briefly re-anaesthetized (sodium methohexitone, 40 mg kg⁻¹) and had an intra-arterial (abdominal aorta via caudal artery) and 3 intravenous (left external jugular) catheters implanted. The catheters were tunnelled subcutaneously and were exteriorized at the same point as the probe wires. The catheters ran through a flexible spring (attached to the harness) supported by a counterbalanced gantry. Animals were left to recover overnight with free access to food and water.

The following day continuous recordings were made of mean blood pressure, heart rate and carotid Doppler shift signals. Although the latter do not give an absolute measure of volume flow, changes in the Doppler shift signal are linearly related to changes in volume flow (Haywood *et al.*, 1981; Wright *et al.*, 1987), and are referred to as flow in the text. Changes (%) in vascular resistances were calculated from the changes in mean blood pressure and

Doppler shift signals, relative to baseline (Haywood *et al.*, 1981; Gardiner *et al.*, 1988).

On the basis of pilot experiments we found that vasopressin administered at 0.3 nmol min⁻¹ had no significant effects on mean blood pressure or heart rate whereas a 10 fold higher dose affected both these variables. Therefore, animals received vasopressin (60 nmol ml⁻¹, 0.3 ml h⁻¹, i.e., 0.3 nmol min⁻¹) for 60 min, followed by a 60 min recovery period. Thereafter vasopressin (600 nmol ml⁻¹, 0.3 ml h⁻¹ i.e., 3 nmol min⁻¹) was administered for 20 min (by which time variables were steady) before (+)-(CH₂)₅Tyr(Et)DAVP (a V₁-receptor antagonist, Manning & Sawyer, 1986) was given (10 µg kg⁻¹, bolus) while the vasopressin infusion was continued. This dose of the V₁-receptor antagonist blocked the pressor actions of vasopressin (10 pmol) for at least 30 min. After a further 20 min, the vasopressin infusion was stopped and recordings continued for an additional 20 min.

The protocol for DDAVP administration involved 60 min infusions followed by 60 min recovery periods with the lower dose (0.3 nmol min⁻¹) being given before the higher dose (3 nmol min⁻¹). Animals were randomized so they received vasopressin on one day and DDAVP on the other experimental day.

From preliminary examination of the experimental traces, measurement points for analysis were chosen at 10, 30, and 60 min during and after vasopressin infusion at 0.3 nmol min⁻¹ and for both doses of DDAVP. For vasopressin infusion at 3 nmol min⁻¹, measurements were made at 5 and

Table 2 Changes in cardiovascular variables during and after infusion of vasopressin (0.3 nmol min⁻¹) in conscious, Long Evans rats

	Vasopressin infusion					
	10 min	30 min	60 min	10 min	30 min	60 min
Heart rate (b min ⁻¹)	6 ± 9	-11 ± 9	-5 ± 10	-5 ± 9	-4 ± 9	0 ± 10
Mean blood pressure (mmHg)	2 ± 1	1 ± 2	2 ± 2	0 ± 1	-2 ± 2	-1 ± 2
Left carotid flow (%)	-6 ± 5	-4 ± 7	-7 ± 3	3 ± 6	14 ± 10	11 ± 7
Right carotid flow (%)	1 ± 7	2 ± 6	-11 ± 4	6 ± 8	20 ± 8	15 ± 10
Left carotid resistance (%)	10 ± 5	9 ± 9	13 ± 7	-2 ± 5	-9 ± 7	-8 ± 5
Right carotid resistance (%)	4 ± 6	3 ± 7	17 ± 5*	-1 ± 7	-15 ± 6*	-10 ± 7*

Values are mean ± s.e.mean (*n* = 8).

* *P* < 0.05 for change relative to baseline (Friedman's test).

20 min after the start of the infusion, 5 and 20 min after administration of the V_1 -receptor antagonist and 5 and 20 min following cessation of the infusion. In addition, attention was paid to the acute changes associated with any intervention (see results). The substances used (vasopressin, Bachem, U.S.A.; DDAVP, Ferring, Sweden and (+)-(CH₂)₅-Tyr(Et)DAVP [1-(β -mercaptr- β , β -cyclopentamethylenepropionic acid),2-O-ethyl-tyrosine,8-D-arginine]vasopressin, Peninsula Laboratories, U.S.A.) were dissolved in isotonic saline; in the case of the V_1 -receptor antagonist the initial solution was made in glacial acetic acid.

Data were subjected to non-parametric analysis of variance (Theodorsson-Norheim, 1987). Where effects are mentioned in the text, then the differences are statistically significant at $P < 0.05$.

Results

Table 1 shows baseline values for the measured cardiovascular variables. The lower rate of infusion of vasopressin ($0.3 \text{ nmol min}^{-1}$) had no effects on mean blood pressure, heart rate or common carotid flows (Table 2). However, there was a tendency for calculated common carotid vascular resistance to increase, and this reached significance in the right common carotid vascular bed (Table 2). There was a vasodilatation in this same vascular bed following cessation of vasopressin infusion (Table 2).

Infusion of vasopressin at the higher rate (3 nmol min^{-1}) caused changes in all measured and calculated variables (Table 3). The decreases in common carotid flows were coincident with the increase in mean arterial blood pressure (Figure 1). Administration of the V_1 -receptor antagonist caused a rapid abolition of the effects of vasopressin on mean blood pressure and heart rate (Figure 2, Table 3), but under these conditions there were increases in

Figure 1 Cardiovascular changes at the onset of infusion of vasopressin (3 nmol min^{-1}) in a conscious, Long Evans rat instrumented with pulsed Doppler probes around both common carotid arteries.

common carotid blood flows (above baseline) accompanied by vasodilatations (Table 3). These effects were persistent, such that 40 min after the administration of the V_1 -receptor antagonist (i.e., 20 min after cessation of vasopressin infusion) there were still reductions in common carotid vascular resistances, at that time accompanied by a small fall in mean arterial pressure (Table 3).

Neither the lower nor the higher rate of infusion of DDAVP had any significant effects on the measured cardiovascular variables.

Discussion

In the present study we found that during infusion of vasopressin, increases in common carotid blood flow were only seen in the presence of a V_1 -receptor

Table 3 Changes in cardiovascular variables during and after infusion of vasopressin ($3.0 \text{ nmol min}^{-1}$) in conscious, Long Evans rats

	<div style="text-align: center;"> V_1-receptor antagonist ↓ Vasopressin infusion </div>					
	5 min	20 min	25 min	40 min	5 min	20 min
Heart rate (b min^{-1})	$-38 \pm 13^*$	$-77 \pm 15^*$	4 ± 10	-1 ± 12	-5 ± 13	-4 ± 11
Mean blood pressure (mmHg)	$14 \pm 4^*$	$26 \pm 4^*$	0 ± 3	$-7 \pm 3^*$	-3 ± 2	$-6 \pm 3^*$
Left carotid flow (%)	$-17 \pm 5^*$	$-24 \pm 7^*$	$21 \pm 5^*$	$21 \pm 9^*$	10 ± 5	$14 \pm 5^*$
Right carotid flow (%)	$-22 \pm 7^*$	$-30 \pm 8^*$	$31 \pm 17^*$	20 ± 19	20 ± 15	$23 \pm 16^*$
Left carotid resistance (%)	$46 \pm 18^*$	$77 \pm 22^*$	$-18 \pm 5^*$	$-21 \pm 7^*$	$-11 \pm 3^*$	$-17 \pm 4^*$
Right carotid resistance (%)	$59 \pm 12^*$	$98 \pm 21^*$	$-17 \pm 7^*$	-12 ± 9	-12 ± 8	$-15 \pm 9^*$

Twenty min into the infusion a V_1 -receptor antagonist ((+)-(CH₂)₅Tyr(Et)DAVP) was administered. Values are mean $\pm$ s.e.mean ($n = 8$).

* $P < 0.05$ for change relative to baseline (Friedman's test).

Figure 2 Cardiovascular effects of administering a V_1 -receptor antagonist ((+)-(CH₂)₅Tyr(Et)DAVP) in a conscious, Long Evans rat that had been infused with vasopressin (3 nmol min⁻¹ for 20 min).

antagonist. In addition, increases in common carotid blood flow following vasopressin infusion were only seen when there had been decreases during the infusion. Common carotid vasodilatation was never seen during or after infusion of DDAVP (a V_2 -receptor agonist).

Although we infused doses of vasopressin sufficiently low as to cause no change in mean blood pressure or heart rate, only decreases in common carotid blood flow were observed during the infusions. These observations do not lend support to the proposition that 'increased levels of vasopressin during hemorrhage and septic shock could favour the redistribution of blood from the periphery to the cerebral circulation and help to maintain cerebral blood flow' (Katusic *et al.*, 1984). Since Katusic & Krstic (1987) subsequently found that vasopressin caused endothelium-independent contractions of common carotid arteries from rats, they modified the earlier proposal (Katusic *et al.*, 1984) by suggesting that a greater contractile response of renal than of common carotid arteries to vasopressin would favour cerebral perfusion when circulating vasopressin levels were raised. However, even under conditions in which sufficient vasopressin has been infused to cause substantial changes in cardiovascular variables (Bennett *et al.*, 1988) we observed only a modest reduction in renal blood flow possibly due to concurrent activation of renal vasodilator

mechanisms (Hofbauer *et al.*, 1983; Walker *et al.*, 1988). Moreover, in our previous study, although the changes in blood pressure and heart rate were similar to those seen with infusion of the higher dose of vasopressin in the present experiments, the increase in renal vascular resistance was only about half the increase in common carotid vascular resistance seen here.

While we know of no evidence from *in vivo* studies in conscious rats that increases in circulating levels of vasopressin favour cerebral perfusion, it is theoretically possible that monitoring common carotid blood flow could be misleading, since these vessels supply both intracranial and extracranial vascular beds. Thus, if vasopressin caused concurrent but directionally opposite, changes in internal and external carotid vascular resistances, for example, this peptide might favour brain perfusion, specifically. At first sight the finding that vasopressin causes constriction of basilar arteries of the rat (Hermsmeyer & Rusch, 1984; Webb *et al.*, 1987) is against this possibility. But it is feasible that differential changes in more distal resistances could augment brain perfusion, even in the face of constriction of the basilar artery, in a way analogous to changes in the proportional perfusion of cortical and medullary portions of the kidney accompanying renal arterial constriction. Indeed, Kozniowska & Skolasinska (1982) found that vasopressin could increase cerebral blood flow in anaesthetized rats. However, these investigators administered vasopressin as bolus doses (into the internal carotid artery) and measured cerebral blood flow (by ¹³³Xe clearance) between 3–4 min later, i.e., at a time when mean blood pressure had returned to control levels. Thus it is possible there had been reductions in cerebral blood flow during the pressor phase following vasopressin administration, and the cerebral vasodilatation observed by Kozniowska & Skolasinska (1982) could represent the same phenomenon as the common carotid vasodilatation, seen in the present study, following infusion of the lower dose of vasopressin, or during infusion of the higher dose of vasopressin in the presence of a V_1 -receptor antagonist.

The serendipitous observation that significant vasodilatation following the end of the infusion of the lower dose of vasopressin only occurred on the same side as that showing significant vasoconstriction during the infusion could indicate that the vasodilator mechanism was triggered by the prior vasoconstriction, or was initially coincident with the latter, but more persistent. The marked common carotid hyperaemia and vasodilatation seen during and after infusion of the higher dose of vasopressin in the presence of a V_1 -receptor antagonist means these phenomena were not dependent on continued V_1 -receptor stimulation, but these observations do

not permit distinction between changes in cerebral metabolism (Kozniowska & Skolasinska, 1982) or persistence of V_1 -receptor-triggered, or non-specific, vasodilator mechanisms as possible causes. While further analysis of these phenomena will require complex experimentation, it is clear from the present results that the most striking effects of vasopressin on common carotid blood flow were seen when its V_1 -receptor actions were antagonized. A similar conclusion, but regarding the effects of vasopressin on total peripheral resistance, was reached by Walker (1986). He demonstrated that a systemic vasodilator effect of vasopressin was apparent even if changes in mean blood pressure were prevented by pretreat-

ment with a V_1 -receptor antagonist. Furthermore, Walker (1986) showed the vasodilatation was not influenced by inhibition of cyclo-oxygenase, although he did not exclude the possible involvement of V_2 -receptors. From the present results, showing that the V_2 -receptor agonist, DDAVP, did not cause common carotid vasodilatation, it seems unlikely that V_2 -receptors mediated the vasodilator effects seen with infusion of the higher dose of vasopressin in the presence of V_1 -receptor antagonism. Hence the latter effect seems to be different from the systemic V_2 -receptor-mediated vasodilatation reported in conscious dogs (Liard, 1988).

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