Vasopressin and stress-induced antinociception in the mouse

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- 1 Arginine vasopressin produced antinociception in the hot-plate test after intracerebroventricular injection ($0.5 \mu\text{g}$) and in the acetic acid abdominal constriction test after intraperitoneal injection (0.1 mg kg^{-1}).
- 2 The antinociception produced by arginine vasopressin was sensitive to deamino(CH_2)₅Tyr(Me) arginine vasopressin ($0.5 \mu\text{g}$ i.c.v.; 0.1 mg kg^{-1} i.p.) but not to naloxone ($5 \mu\text{g}$ i.c.v.; 2 mg kg^{-1} i.p.)
- 3 Arginine vasopressin when administered by the intracerebroventricular route, but not by the intraperitoneal route, produced characteristic behaviour which was sensitive to deamino(CH_2)₅Tyr(Me) arginine vasopressin ($0.5 \mu\text{g}$, i.c.v.).
- 4 A 3 min swim at 20°C produced antinociception on the hot-plate which was sensitive to naloxone (0.4 mg kg^{-1} , i.p.) but not to deamino(CH_2)₅Tyr(Me) arginine vasopressin ($0.5 \mu\text{g}$, i.c.v.).
- 5 The reduction in the number of acetic acid-induced abdominal constrictions produced by a 30 s swim at 30°C was not sensitive to either naloxone (2 mg kg^{-1} , i.p.) or deamino(CH_2)₅Tyr(Me) arginine vasopressin (0.1 mg kg^{-1} , i.p.).
- 6 Arginine vasopressin, at high doses, is antinociceptive in the mouse but does not appear to mediate stress-induced antinociception in this species.

Introduction

Stress-induced antinociception (SIA) is a complex phenomenon and the characteristics of a particular model of SIA depend not only on the animal species but also on the nature of the stress and the intensity of noxious stimulus used to assess the antinociception.

Several neurotransmitters, including acetylcholine, noradrenaline, histamine and 5-hydroxytryptamine are capable of producing antinociception and these have all been implicated in various models of SIA (Hutson *et al.*, 1984; Hart & Yadav, 1985; Hart & Oluyomi, 1986; 1987). Much attention has been focused on the involvement of endogenous opioid peptides in SIA (Lewis *et al.*, 1984) and, in addition, it has been suggested that another peptide, namely vasopressin, may also be involved (Bodnar *et al.*, 1980; Hamamura & Onaka, 1987).

The antinociceptive activity of lysine vasopressin, after intraventricular or subcutaneous administration, was demonstrated by Berntson & Berson (1980) in the rat, using radiant heat on the tail as the noxious stimulus; the antinociception was not naloxone-sensitive. Support for a physiological role for vasopressin in nociception was provided by the report that Brattleboro rats have a lowered threshold to foot-shock and a reduced level of antinociception after a cold water swim (Bodnar *et al.*, 1980). Further evidence for the involvement of vasopressin in SIA was provided by Hamamura & Onaka (1987) who demonstrated that the vasopressin antagonist, deamino(CH_2)₅Tyr(Me) arginine vasopressin, inhibited a naloxone insensitive model of SIA in the rat at a dose which also reduced the antinociception produced by exogenous arginine vasopressin. This latter observation was in contrast to the results of Caldwell *et al.* (1987) who found that deamino(CH_2)₅Tyr(Me) arginine vasopressin did not antagonize the antinociceptive effects of arginine-vasopressin in the mouse.

In the present study we have demonstrated the antinociceptive activity of arginine vasopressin in the mouse, using two different noxious stimuli. The characteristics of this antinociception have been examined by determining its sensitivity to naloxone and to the selective vasopressin antagonist deamino(CH_2)₅Tyr(Me)AVP (Kruszynski *et al.*, 1980). This antagonist has been employed to investigate the involvement of arginine vasopressin in SIA in the mouse by use of both a naloxone-sensitive and naloxone-insensitive model of SIA.

Methods

Male LACA mice (25–35 g), housed at $22 \pm 2^\circ\text{C}$ with illumination from 07 h 00 min to 21 h 00 min and with food and water *ad libitum*, were allowed to acclimatize for at least 90 min in the laboratory prior to experimentation between 11 h 00 min and 17 h 00 min.

Nociception

Two methods were used to assess the degree of antinociception produced by arginine vasopressin or by stress. In the hot-plate test the mouse was placed on a surface maintained at 56°C for 45 s or until lifting or shaking of a hind paw was observed; movement of mice on the hot plate was limited by a plexiglass cylinder (26 cm high and 20 cm diameter). An initial reaction time was obtained for each mouse and only those responding within 10 s were used. Potential antinociceptive agents were injected by the intracerebroventricular (i.c.v.) route 30 min after the screen reaction time had been measured and the effect of the agent was determined 10 min later. When antagonists were studied they were administered (i.c.v.) 30 min after the screen reaction time and 10 min before the antinociceptive agent. The abdominal constriction test involved the intraperitoneal (i.p.) injection of acetic acid (0.6% w/v, 0.01 ml g^{-1}) with the abdominal constrictions counted between 10 and 20 min after the injection of the acetic acid. Potential antinociceptive agents were administered 15 min before the acetic acid and antagonists 10 min before the antinociceptive agent, both by the i.p. route.

In the investigation of arginine vasopressin-induced antinociception, i.c.v. administration was used in the hot-plate test as this method involves a supraspinal response whilst the i.p. route was used for the abdominal constriction test which is sensitive to antinociceptive agents which act peripherally (Smith *et al.*, 1982). When investigating the possible involvement of opioids, naloxone was administered by the same route as the arginine vasopressin.

Stress-induced antinociception

Two models of SIA were investigated which differed in the intensity of the stress, the method of assessment of antinociception and in their sensitivity to naloxone. These have

been described previously (Hart *et al.*, 1983) and are summarised as follows:

- (1) Mice were stressed by being placed in a water bath (57 cm × 30 cm, depth of 19 cm) at 20°C for 3 min. Hot-plate reaction times were determined three times: 30 min before the administration of antagonist or saline; 10 min later, which was immediately before the stress, and 2 min after the stress. The 2 min delay between the end of the stress and the measurement of a reaction time was occupied by the drying of the mouse. SIA was expressed as the difference between the post and pre-stress values for reaction times. Mice receiving saline were treated in the same manner apart from the swim. Naloxone was administered by the i.p. route and deamino(CH₂)₅Tyr(Me)AVP was injected by the i.c.v. route.
- (2) Mice were placed in the water bath for 30 s at a water temperature of 30°C with antagonists or saline being injected (i.p.) 15 min before the stress. On removal from the water the mice were injected with acetic acid (as described above) and abdominal constrictions counted for 10 min after a delay of 10 min.

Drugs

Morphine hydrochloride, arginine vasopressin and the vasopressin antagonist [1-(β-mercapto-β,β-cyclopentomethylene propionic acid), 2-(O-methyl) tyrosine] arginine vasopressin (deamino(CH₂)₅Tyr(Me)AVP) were obtained from Sigma Ltd., naloxone hydrochloride was a gift from Endo Laboratories. In most experiments using the hot-plate, drugs or saline were administered by the i.c.v. route by a modification of the method described by Haley & McCormick (1957) with the injection volume not exceeding 5 µl. Coded solutions were used throughout the investigation with at least six mice in each experimental group. The investigator was not allowed access to the code until after the completion of the experiment.

Analysis

Results from both the hot-plate test (change in reaction times) and from the abdominal constriction test (number of constrictions in 10 min period) are expressed as medians and comparisons between groups has been assessed by the appropriate test; either the Kruskal-Wallis test or the Mann-Whitney test.

Results

Vasopressin-induced antinociception

Arginine vasopressin (0.5, 1 µg) injected i.c.v. produced a small but significant increase in reaction times of mice on the hot-plate (Table 1). The antinociception produced by 0.5 µg arginine vasopressin was abolished completely by the vasopressin antagonist deamino(CH₂)₅Tyr(Me)AVP (0.5 µg, i.c.v.) but unaffected by a dose of naloxone (5 µg, i.c.v.) which produced significant inhibition (approximately 90%) of morphine (5 µg, i.c.v.)-induced antinociception. In contrast, the vasopressin antagonist did not reduce antinociception due to morphine.

A similar pattern of results were obtained in the abdominal constriction test (Table 2). In mice treated with saline, the administration of acetic acid caused 26.5 (IQR 25–29) constrictions which was significantly reduced by both arginine vasopressin (0.1 mg kg⁻¹, i.p.) and morphine (2.5 mg kg⁻¹, i.p.). The effect of arginine vasopressin was inhibited by deamino(CH₂)₅Tyr(Me)AVP (0.1 mg kg⁻¹, i.p.) (*P* < 0.001) but not by naloxone (2 mg kg⁻¹, i.p.) and the reduction in abdominal constrictions produced by morphine was sensitive to naloxone but not to the vasopressin antagonist.

Stress-induced antinociception

In mice receiving saline (5 µl i.c.v.) a 3 min swim at 20°C increased the reaction time on the hot-plate by a median of 12.25 s (Table 3). This SIA was unaffected by deamino(CH₂)₅Tyr(Me)AVP at a dose (0.5 µg i.c.v.) which had

Table 1 Change in reaction time of mice on the hot-plate 10 min after administration of morphine or arginine vasopressin alone, or in presence of antagonist

Drug	Dose (µg)	Median (s)	IQR
Saline		0.5	0–0.5
Morphine	5	32.5*	29–39
Naloxone	5	0	0–0.5
Vasopressin	0.5	4*	4–5
	1	5*	4–6
d(CH ₂) ₅ Tyr(Me)AVP	0.5	0	0–0.5
Saline +			
Vasopressin	0.5	3.75	3–4.5
d(CH ₂) ₅ Tyr(Me)AVP +	0.5		
Vasopressin	0.5	0*	0–0.5
Naloxone +	5		
Vasopressin	0.5	4	4–4.5
d(CH ₂) ₅ Tyr(Me)AVP +	0.5		
Morphine	5	31	30–37
Naloxone +	5		
Morphine	5	3*	2.5–4

All injections were by the i.c.v. route. IQR = interquartile range. d(CH₂)₅Tyr(Me)AVP = deamino(CH₂)₅Tyr(Me) arginine vasopressin.

**P* < 0.001 against appropriate control by Kruskal-Wallis test.

Table 2 Median number of abdominal constrictions recorded 10–20 min after i.p. administration of acetic acid

Treatment	Dose (mg kg ⁻¹)	Median	IQR
Saline		26.5	25–29
Vasopressin	0.1	12.5*	12–14
Morphine	2.5	8*	6–9
d(CH ₂) ₅ Tyr(Me)AVP	0.1	27	24–29
Naloxone	2	28.5	27–30
Saline +			
Vasopressin	0.1	13.5	12–16
d(CH ₂) ₅ Tyr(Me)AVP +	0.1		
Vasopressin	0.1	25.5#	24–26
d(CH ₂) ₅ Tyr(Me)AVP +	0.1		
Morphine	2.5	7	7–8
Naloxone +	2		
Vasopressin	0.1	11.5	9–14
Naloxone +	2		
Morphine	2.5	22*	20–23
Saline + swim		8.5*	5–9
d(CH ₂) ₅ Tyr(Me)AVP + swim	0.1	8.5	7–9
Naloxone + swim	2	9.5	8–10

Swim was for 30 s at 30°C. Morphine and vasopressin were administered 15 min before the acetic acid and antagonists 10 min before the agonist. All injections were by the i.p. route. IQR = interquartile range. d(CH₂)₅Tyr(Me)AVP = deamino(CH₂)₅Tyr(Me) arginine vasopressin.

**P* < 0.01 against appropriate control by Kruskal-Wallis test. #*P* < 0.001 against saline + vasopressin control by Mann-Whitney test.

Table 3 Difference between post-swim and pre-swim reaction times on the hot-plate (i.e. SIA) in mice treated with saline, deamino(CH₂)₅Tyr(Me) arginine vasopressin (d(CH₂)₅Tyr(Me)AVP) or naloxone

Treatment	Dose	Median (s)	IQR
Saline	5 µl i.c.v.	12.25	10–13
d(CH ₂) ₅ Tyr(Me)AVP	0.5 µg i.c.v.	12	12–13
Saline	10 ml kg ⁻¹ i.p.	11	8–12
Naloxone	0.4 mg kg ⁻¹ i.p.	6*	3–8.5
	4 mg kg ⁻¹ i.p.	1**	–2–3

Post-swim reaction time was measured 2 min after the 3 min swim at 20°C. In mice treated with saline but not swum the median increase in reaction time was 0.5 s. IQR = interquartile range.

P* < 0.05; *P* < 0.01 by Kruskal-Wallis test.

inhibited the antinociception produced by arginine vasopressin on the hot-plate. The involvement of endogenous opioids in this model of SIA was confirmed by the reduced antinociception observed in the mice treated with naloxone.

Stressed mice (30 s swim at 30°C) responded to acetic acid with a reduced number of abdominal constrictions i.e. 8.5 compared to 26.5 in unstressed animals ($P < 0.01$). In this case the SIA was unaffected by prior treatment of the mice with either deamino(CH₂)₅Tyr(Me)AVP or naloxone at doses that reduced the effects of arginine vasopressin and morphine respectively in the abdominal constriction test (Table 2).

Behaviour

Although not estimated quantitatively in this study, animals injected with arginine vasopressin were observed carefully for any gross changes in behaviour. The i.c.v. administration of arginine vasopressin elicited an immediate and consistent pattern of behaviour involving excessive grooming and alternate hind limb scratching directed at the head. Between 4 min and 6 min after injection of vasopressin, vocalisation occurred which continued for more than an hour whilst the characteristic grooming behaviour lasted for 10 min. Both the grooming behaviour and vocalisation responses to arginine vasopressin were inhibited by deamino(CH₂)₅Tyr(Me)AVP (0.5 µg, i.c.v.) which alone produced no marked changes in behaviour. The behaviour of mice given arginine vasopressin by the intraperitoneal route did not differ from that of animals treated with saline.

Discussion

The present study confirms that arginine vasopressin is antinociceptive in the mouse and, significantly, demonstrates antinociception occurring after both i.c.v. and i.p. administrations of this peptide. Previous workers have failed to show antinociception by the i.c.v. route. Tulunay *et al.* (1982) used doses of between 0.01 and 0.1 µg per mouse and did not observe antinociception using a radiant heat tail-flick method whilst Berkowitz & Sherman (1982) found that 1–10 µg kg⁻¹ was effective intravenously but not intracerebroventricularly in the abdominal constriction test. The positive results obtained in the present study may reflect the higher doses of arginine vasopressin, 0.5 µg and 1 µg per mouse, which were employed. Caldwell *et al.* (1987) showed that arginine vasopressin was antinociceptive in mice on the hot-plate with doses of 1 µg and 4 µg administered intracisternally. In contrast to the results of Berkowitz & Sherman (1982), studies in the rat (Kordower *et al.*, 1982) showed lysine vasopressin to be active by the i.c.v. route (500 ng) but not after subcutaneous injection (1500 ng).

Berkowitz & Sherman (1982) suggested that arginine vasopressin may act peripherally in producing antinociception and this would appear to be supported by the present finding that intraperitoneal arginine vasopressin (0.1 mg kg⁻¹) reduced the number of abdominal constrictions produced by acetic acid. However, no firm conclusions can be drawn about the antinociceptive site of action of arginine vasopressin from these experiments; the i.c.v. dose was high and may have entered the peripheral circulation whilst it is possible, but unlikely, that the intraperitoneally administered peptide would have penetrated the brain. Banks & Kastin (1987), in a review of peptide movement across the blood:brain barrier, concluded that peripherally administered arginine vasopressin is unlikely to enter the brain whilst the peptide is cleared rapidly from the cerebrospinal fluid after central administration. The spinal cord does not appear to be the site of action of arginine vasopressin in the rat for intrathecal vasopressin was not antinociceptive and lesions of the paraventricular nucleus, which

lowered levels of vasopressin in the cord, did not affect tail-flick latencies (Millan *et al.*, 1984).

Antinociception induced by arginine vasopressin was attenuated by the selective antagonist deamino-(CH₂)₅Tyr(Me)AVP administered 10 min prior to the peptide whereas Caldwell *et al.* (1987) reported no inhibition of antinociception in the mouse when the antagonist was administered at the same time as arginine vasopressin by the intracisternal route. Hamamura & Onaka (1987) found that the increase in the tail-flick latency in the rat induced by the intravenous administration of arginine vasopressin was sensitive to deamino(CH₂)₅Tyr(Me)AVP given intravenously 1 h before the vasopressin. It would seem that the lack of activity observed by Caldwell *et al.* (1987) may be explained in the timing of the administration of the antagonist. Kruszynski *et al.* (1980) reported that the pA₂ for deamino-(CH₂)₅Tyr(Me)AVP against the pressor activity of arginine vasopressin was 8.62 whilst it had weak antidiuretic activity and it may thus be considered a selective antagonist of V₁ vasopressin receptors (Manning & Sawyer, 1984). The inhibition by deamino(CH₂)₅Tyr(Me)AVP of both the behavioural activities induced by arginine vasopressin and the antinociception suggests that in the mouse the receptors involved are probably of the same V₁ class. In the present study the vasopressin antagonist had no effect on morphine induced antinociception nor did naloxone affect antinociception due to arginine vasopressin.

This finding that arginine vasopressin-induced antinociception does not involve opioid pathways confirms the results of others in mice (Berkowitz & Sherman, 1982) and rats (Berntson & Berson, 1980; Berson *et al.*, 1983; Watkins *et al.*, 1986). The lack of interaction between opioid- and vasopressin-induced antinociception is surprising in view of the reported co-existence of vasopressin and dynorphin in neurones in the brain (Cox *et al.*, 1982) and the potentiation of morphine antinociception observed with 10–100 ng arginine vasopressin administered intracerebroventricularly to the mouse (Tulunay *et al.*, 1982). This could be studied further by investigating whether there is any interaction between arginine vasopressin and a κ -opioid agonist.

The model of SIA involving a 3 min swim at 20°C with antinociception assessed on the hot-plate is sensitive to low doses of naloxone and to a selective δ -opioid receptor antagonist (Hart *et al.*, 1983; 1985). Endogenous opioids are thus important in this model and there is good evidence for the additional involvement of cholinergic and adrenergic pathways (Hart & Yadav, 1985) but the present investigation shows that arginine vasopressin is unlikely to have a significant role. When the duration of the swim is reduced to 30 s and water temperature increased to 30°C the resulting antinociception is too mild to be measured on the hot-plate but can be assessed as a reduction in the number of constrictions induced by acetic acid. This SIA is not attenuated by high doses of naloxone (Hart *et al.*, 1983), as confirmed in the present study, and was unaffected by the vasopressin antagonist at a dose which produced significant inhibition of the antinociceptive action of vasopressin. The demonstration that arginine vasopressin is not involved in SIA in the mouse, in contrast to its well documented role in the rat (Bodnar *et al.*, 1980; Hamamura & Onaka, 1987), further emphasizes that the characteristics of SIA depend upon the species and the method of assessment of antinociception (Kelly, 1982).

In conclusion, the results of this study show that arginine vasopressin is antinociceptive in the mouse in both the hot-plate and abdominal constriction tests but that there is no evidence for its involvement in either naloxone-sensitive or naloxone-insensitive models of SIA.

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