Central effect of the enkephalin analogue FK-33824 on vasopressin secretion in conscious sheep

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Abstract. The role of opioids in the regulation of arginine vasopressin release from the posterior pituitary is a subject of controversy. In the present study, we examined the effects of central administration of met-enkephalin, leu-enkephalin, the enkephalin analogue FK-33824, and the opiate antagonist naloxone, and the effects of systemic administration of met-enkephalin and FK-33824 on AVP secretion in conscious normal sheep. Intracerebroventricular infusion of FK-33824 significantly increased the plasma concentration of immunoreactive AVP in a dose-dependent manner, but met-enkephalin, leu-enkephalin and naloxone failed to change plasma concentration of AVP. Intravenous infusion of met-enkephalin and FK-33824 also failed to change plasma concentration of AVP. The opiate antagonist naloxone given both centrally and systemically attenuated the increase in plasma concentration of AVP induced by FK-33824. We conclude that basal AVP release is stimulated by central administration of FK-33824.

Development of a variety of synthetic opioid analogues has enabled us further to investigate the role of opioids in the regulation of vasopressin secretion from the posterior pituitary. FK-33824, [D-Ala², N-Me-Phe⁴, Met(O)³-ol]-enkephalin (DAMME), is a potent and long-lasting synthetic enkephalin analogue in analgesic activity (Roemer et al. 1977). It has been shown in humans that systemic administration of FK-33824 inhibits the secretion of arginine vasopressin (Brownell et al. 1980; Grossman et al. 1980). However, its effect by central administration of FK-33824 is unknown, and so far very few studies using the species sheep have been undertaken to evaluate how opioids affect AVP secretion.

In the present study, we examined the effects of intracerebroventricular (icv) infusion of FK-33824, met-enkephalin, leu-enkephalin and the opiate antagonist naloxone, and the effects of intravenous infusion of met-enkephalin and FK-33824 on plasma concentration of AVP in conscious normal sheep.

Material and Methods

Animal preparation

Seven oophorectomized Merino ewes, weighing 35–45 kg, were used. Bilateral carotid arterial loops were prepared surgically and guide tubes were implanted towards the lateral cerebral ventricles (icv) at least 3 months before experimentation (Wang et al. 1987). The animals were housed in individual metabolism cages and handled regularly to accustom them to experimental procedures. An indwelling cannula was inserted into the jugular vein at least 24 h before each experiment. All the experiments were carried out in the same time of morning and animals were allowed to have a recovery period of 5–7 days after each experiment.

Experimental protocol

In a series of experiments, met-enkephalin (3.3 µg/min for 30 min, N = 7), leu-enkephalin (3.3 µg/min for 30 min, N = 4), FK-33824 (0.08, 0.3 and 0.8 µg/min for 30 min, N = 4), and naloxone (8.3 µg/min for 30 min, N = 4) were infused into the lateral cerebral ventricle at a rate of 20 µl/min. Infusions of artificial cerebrospinal fluid (CSF)
(20 µl/min, N = 5) (Mouw et al. 1974) were used as a vehicle control.

Intracerebroventricular infusion of FK-33824 (0.3 µg/min for 30 min) together with naloxone (8.3 µg/min for 30 min) (N = 6) was given, and iv infusion of naloxone (83 µg/min for 30 min) was given 10 min after the start of icv infusion of FK-33824 (0.3 µg/min for 30 min) (N = 4).

Met-enkephalin (83 µg/min for 30 min, N = 5), FK-33824 (8 µg/min for 30 min, N = 5) at a rate of 0.5 ml/min and physiological saline (0.5 ml/min for 30 min, N = 4) were infused iv.

All the peptides (from Peninsula Laboratories, Belmont, CA) and naloxone (from E. I. Du Pont Pharmaceuticals, Wilmington, DE) were dissolved in artificial CSF for icv infusion and in physiological saline for iv infusion. Blood samples (5 ml each) for AVP assay were taken from a needle positioned in the carotid artery 10 min before the infusion, and then at 10, 20, 30, 40, 60 and 90 min after commencement of the infusions or hemorrhage.

Radioimmunoassay
Plasma AVP concentration was measured by radioimmunoassay. Antisera raised in rabbits against AVP was used in the assay. Blood samples were collected into chilled tubes containing heparin (10⁶ IU/l). Plasma (1.5 ml) was extracted using an acetone extraction procedure (Wang et al. 1987). The extracts were assayed in triplicate. The antibody cross-reacted less than 0.1% with oxytocin and less than 0.2% with vasotocin. The antibody was used at a final dilution of 1:250,000. The sensitivity of the assay was 0.4 pmol/l. The intra-assay coefficient of variation was 4.4% (N = 8) and inter-assay variation was 8% (N = 8).

Statistics
Results are expressed as the mean ± standard error of the mean (SEM). The significance of changes was determined using the Wilcoxon paired test.

Results
Fig. 1 shows that intracerebroventricular infusion of FK-33824 produced a dose-related elevation in plasma concentration of AVP. At the dose of 0.08 µg/min, plasma concentration of AVP rose from a pre-infusion value of 0.7 ± 0.1 pmol/l to a peak value of 2.6 ± 0.5 pmol/l at 90 min (P < 0.05). At the dose of 0.3 µg/min, plasma concentration of AVP rose from 1.0 ± 0.2 to 7.6 ± 1.8 pmol/l at 90 min (P < 0.05). At the dose of 0.8 µg/min, plasma concentration of AVP rose from 0.7 ± 0.1 to 9.9 ± 4.4 pmol/l at 90 min (P < 0.05). In the control study, there was no change in plasma concentration of AVP after icv infusion of artificial CSF.

Changes in plasma AVP concentration after intracerebroventricular infusion of the enkephalin analogue FK-33824 at 0.08 µg/min (diagonally striped bar, N = 4), 0.3 µg/min (horizontally striped bar, N = 4) and 0.8 µg/min (solid bar, N = 4) and the artificial cerebrospinal fluid (open bar, N = 5). Statistics (*P < 0.05) compare individual values with the pre-infusion values.

Fig. 1.
Changes in plasma AVP concentration after intracerebroventricular infusion of the enkephalin analogue FK-33824 at 0.3 µg/min (●) (N = 4), icv infusion of FK-33824 at 0.3 µg/min together with icv naloxone at 8.3 µg/min (▲) (N = 6) and icv infusion of FK-33824 at 0.3 µg/min plus iv infusion of naloxone 83 µg/min (○) (N = 4). The period of icv infusion is shown by the solid bar and the period of iv infusion by the open bar. Statistics (*P < 0.05) compare individual values with the pre-infusion values.

Both icv infusion and iv infusion of naloxone attenuated the central elevation in plasma concentration of AVP induced by FK-33824, as shown in Fig. 2. Intracerebroventricular infusion of FK-33824 alone increased plasma concentration of AVP from a pre-infusion value of 1.0 ± 0.2 to a peak value of 7.6 ± 1.8 pmol/l at 90 min (P < 0.05). With icv infusion of naloxone together with FK-33824, plasma concentration of AVP rose from 0.7 ± 0.2 to 1.5 ± 0.6 pmol/l at 90 min (P < 0.05), and with iv infusion of naloxone plus icv infusion of FK-33824, plasma concentration of AVP rose from 1.0 ± 0.2 to 2.1 ± 0.4 pmol/l at 90 min (P < 0.05). At 90 min there was a significant difference (P < 0.05) in the treatments between icv infusion of naloxone together with FK-33824 and FK-33824 alone, as well as between iv infusion of naloxone with icv FK-33824 and FK-33824 alone.

Intracerebroventricular infusion of met-enkephalin, leu-enkephalin and naloxone did not change plasma concentration of AVP significantly, as shown in Table 1. Intravenous infusions of met-enkephalin, FK-33824 and physiological saline were also without effect on plasma concentration of AVP.

### Discussion

Many earlier studies in animals have shown that acute administration of morphine can cause an antidiuresis, suggesting it is attributed to stimulation of AVP release from the posterior pituitary (De Bodo 1944; Duke et al. 1951; Halder 1982; Rockhold et al. 1983; Vandeputte-Van Messon & Peeters 1980). This finding was confirmed later by using synthetic opioid peptides (Bisset et al. 1978; Weitzman et al. 1977). Further, the antidiuretic action of opioid peptides could be antagonised by naloxone both intracerebroventricularly and intravenously (Vandeputte-Van Messon & Peeters 1980). Our observation, using the potent synthetic

### Table 1.

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<td>–10</td>
<td>10</td>
<td>20</td>
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<tr>
<td>Met-enkephalin</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.2</td>
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<td>(3.3 µg/min, N = 7)</td>
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<td></td>
<td>0.9 ± 0.2</td>
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<td>0.7 ± 0.2</td>
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<tr>
<td>Leu-enkephalin</td>
<td>1.0 ± 0.3</td>
<td>1.0 ± 0.4</td>
<td>1.5 ± 0.5</td>
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<td>(3.3 µg/min, N = 4)</td>
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<td>1.4 ± 0.5</td>
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<td>1.5 ± 0.5</td>
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<tr>
<td>Naloxone</td>
<td>0.8 ± 0.1</td>
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<td>(8.3 µg/min, N = 4)</td>
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<td>0.7 ± 0.2</td>
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enkephalin analogue FK-33824 centrally administered in the conscious sheep, is in agreement with the previous findings in animals that opioids could stimulate AVP release and that naloxone could antagonise the stimulation of AVP release produced by opioids (Bisset et al. 1978; Rockhold et al. 1983; Vandeputte-Van Messon & Peeters 1980; Weitzman et al. 1977).

The sites at which FK-33824 can be acting are not clear. Immunoreactive opioid peptides and opiate receptors are located in the magnocellular division of the supraoptic nucleus and the paraventricular nucleus of the hypothalamus (Atweh & Kuhar 1983; Khachaturian et al. 1985). Bisset and colleagues demonstrated in vivo that leu-enkephalin and morphine injected into cerebral ventricles produced a marked antidiuretic action in rats, suggesting a direct stimulation of neurones in the supraoptic or paraventricular nuclei of the hypothalamus (Bisset et al. 1978).

Another possible site of FK-33824 action may be on the complex neurotransmitter system, by mediating neurotransmitter releases and/or activating neurotransmitter turnovers. For example, the cholinergic nerve system is known to stimulate AVP release from the posterior pituitary. It is suggested that the cholinergic stimulation of AVP release is via an activation of nicotinic receptors (Sklar & Schrier 1983). The central administration of FK-33824 may result in an increase in the concentration of acetylcholine in the brain. There is evidence that opioids can stimulate acetylcholine release in some brain tissues (Botticelli & Wurtman 1979).

A less likely site of action may be at the posterior pituitary. Our data demonstrated that systemic administration of FK-33824 and met-enkephalin at the dose 10 times more than that for central administration failed to influence AVP secretion. Several studies have shown that opioids do not affect directly AVP release from the posterior pituitary in vitro (Bicknell et al. 1985; Christensen & Fjalland 1982; Nordmann et al. 1986; Weitzman et al. 1977) although opiate binding sites have been located in the posterior pituitary (Bisset et al. 1978; Simantov & Snyder 1977).

Finally, the regulation of AVP secretion from the posterior pituitary by the opioid systems is rather complex because of the multiple classes of precursors and opioid peptides, different receptor types, and the wide opioid distribution in the brain and other parts of the body (Atweh & Kuhar 1983; Khachaturian et al. 1985). The results of action by opioids on AVP release can be different, probably depending on many factors including the route of administration, the type of receptor involved and the species. Equally, the background activity of AVP release is important as well. Further studies are required to determine the precise sites of action of opioids and the functional characteristics of their action on AVP release in both normal physiological and pathophysiological conditions.

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References


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