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Inhibition of elevated arginine vasopressin secretion in response to osmotic stimulation and acute haemorrhage by U-62066E, a κ-opioid receptor agonist

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1 The effect of kappa (κ) opioid receptor activation on the increase in arginine vasopressin (AVP) secretion evoked by two acute and quite different stimuli (i.e., haemorrhage and osmotic stimulus due to hypertonic saline infusion) were evaluated in conscious Long-Evans rats, by use of U-62066E, a highly selective κ-opioid receptor agonist, and MR2266, an opioid receptor antagonist with some selectivity for κ-receptors.

2 An acute haemorrhage, which reduced the mean blood pressure by approximately 50%, resulted in a large increase in the plasma AVP (pAVP) levels of control rats. However, the administration of U-62066E ($0.2\,mg\,kg^{-1}$ or $2.0\,mg\,kg^{-1}$) reduced the increase due to haemorrhage in a dose-dependent manner. In contrast, concomitant administration of $2.0\,mg\,kg^{-1}$ of MR2266 with U-62066E significantly attenuated the inhibition of pAVP levels produced by U-62066E $2.0\,mg\,kg^{-1}$.

3 Hypertonic saline infusion (5% hypertonic saline solution at a rate of $0.24\,ml\,kg^{-1}\,min^{-1}$ for 10 min) caused the elevation of plasma osmolality (pOsm) from $294.0 \pm 1.6\,mosmol\,kg^{-1}$ to $304.4 \pm 1.9\,mosmol\,kg^{-1}$, simultaneously resulting in a significant increase in pAVP levels from $2.34 \pm 0.28\,pg\,ml^{-1}$ to $4.54 \pm 0.51\,pg\,ml^{-1}$. However, the administration of U-62066E ($0.05\,mg\,kg^{-1}$ or $0.2\,mg\,kg^{-1}$) reduced the osmotically induced increase in pAVP in a dose-dependent manner although pOsm showed the same degree of increase as in controls. In contrast, concomitant administration of $0.2\,mg\,kg^{-1}$ of MR2266 with U-62066E significantly attenuated the inhibition of pAVP levels produced by U-62066E $0.2\,mg\,kg^{-1}$, whereas pOsm showed the same degree of increase as in controls. No significant changes in the mean blood pressure of the respective groups were observed during this experiment.

4 It is suggested that the κ-opioid receptor activation reduces the increase in AVP secretion evoked by these two different stimuli and that the inhibitory involvement occurs in the neural lobe in the process of AVP secretion.

Introduction

It is well known that the release of arginine vasopressin (AVP) is stimulated not only by osmotic stimulus but also by volume depletion i.e. baroreceptor and volume-receptor mediated responses (Aisenbrey *et al.*, 1981; Paller & Linas, 1984). Indeed, acute haemorrhage stimulates the secretion of AVP (Ginsburg & Heller, 1953; Laycock *et al.*, 1979). Baroreflex-mediated changes in sympathetic and cardiovascular function are strongly affected by μ- and δ-opioid receptor activation (Gordon, 1986; Faden & Feuerstein, 1983). However, little has been reported as to whether the κ-opioid receptor can affect the increase in AVP secretion in response to the acute baroreflex. κ-Opioid receptors have been found to be abundant in neurosecretosome preparations (Falke & Martin, 1985; Pesce *et al.*, 1987). An autoradiographic analysis has shown that κ-receptors are concentrated around the neural lobe perimeter (Herkenham *et al.*, 1986). However, specific binding activity of κ-agonists was also observed in the rat hypothalamus, preoptic area, central nucleus of the amygdala, and rostral nucleus of the solitary tract. Carter & Lightman (1985) reported that a κ-receptor agonist, U-50488H, microinjected into the nucleus tractus solitarius, generally accepted as the vasomotor centre for control of AVP secretion by peripheral baroreceptors (Share, 1974) and the site of visceroreceptive afferent termination (Torvitz, 1956), markedly increased plasma AVP. Thus, the evidence strongly suggests that central κ-opioid receptor activation stimulates AVP secretion. On the other hand, it has been reported that the administration of κ-opioid receptor agonists decreases plasma AVP levels in water-deprived rats (Slizgi & Ludens, 1982; Leander, 1983; Leander *et al.*, 1987), and that the suppression of plasma AVP levels might be responsible for the increase in urinary output (Yamada *et al.*, 1989a).

Therefore, the present study was designed to determine whether the effect of a κ-opioid receptor agonist on the response of AVP to two acute and different stimuli (i.e. osmotic stimulus due to hypertonic saline infusion and haemorrhage) which activate the reflex arc through the nucleus tractus solitarius, takes place somewhere in the final common pathway or in the previous separate ones in the process of AVP neurosecretion. A highly selective κ-opioid receptor agonist, U-62066E (Lahti *et al.*, 1985; Mickelson & Lahti, 1984), and an opioid antagonist with some selectivity for the κ-opioid receptor, MR2266 (Jacob & Ramabadram, 1977), were used.

Methods

Long-Evans rats weighing 300–350 g were used. Animals were anaesthetized with sodium pentobarbitone ($50\,mg\,kg^{-1}$, i.p.) and polyethylene catheters were placed in both femoral arteries for measurement of arterial pressure, infusion of hypertonic saline infusion, blood samplings and induction of haemorrhage. Rats were housed with free access to water but without chow in a temperature-controlled room (22 ± 2°C). Experiments were carried out on the following day.

Protocol

On the day following the surgery, blood pressure was monitored by a transducer (Nihon Koden type 101T) coupled to a polygraph (Nihon Koden RM-600) in conscious rats. Blood was sampled over 30 s (B_0 and B_1, each 0.5 ml as EDTA-

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treated whole blood). An equal volume of normal saline was replaced each time.

Haemorrhage After withdrawing blood (B_0), the κ-opioid agonist (U-62066E, 0.2 mg kg^{-1} or 2.0 mg kg^{-1}) or vehicle was administered i.p. The κ-opioid antagonist MR2266 (0.2 mg kg^{-1} or 2.0 mg kg^{-1}) was administered i.p. 5 min after U-62066E or the vehicle. The second sampling of blood (B_1) was performed 20 min after U-62066E or vehicle administration. Thereafter, blood was drawn continuously through a catheter for 5 min at a rate of 1 ml min^{-1} per 300 g rat with a pump (Mastertilex, Cole-Palmer Instrument Co.). The rats were killed by decapitation immediately when mean blood pressure became half of the value just prior to haemorrhage, and the blood was collected for subsequent determination of plasma AVP (pAVP) (B_2) and osmolality (pOsm).

Hypertonic saline infusion Infusion of 5% hypertonic saline solution at a rate of 0.24 ml kg^{-1} min^{-1} for 10 min with a Harvard pump (Harvard Apparatus 973C) was carried out. As a preliminary experiment, the time course of the plasma AVP response to the above hypertonic saline infusion was examined in blood before and at 10, 20, and 30 min after the start of the infusion. As shown in Figure 1, the maximal level of pAVP was observed at 10 min after the start of the infusion. Therefore, for measuring the change in pAVP level in response to hypertonic saline infusion, we chose the 10 min value in the following experiments.

After withdrawing blood (B_0), the κ-opioid agonist (U-62066E, 0.05 mg kg^{-1} or 0.2 mg kg^{-1}) or the vehicle was administered i.p. The κ-opioid antagonist MR2266 (0.05 mg kg^{-1} or 0.2 mg kg^{-1}) was administered i.p. 5 min after U-62066E or vehicle administration. The second sampling of blood (B_1) was performed 20 min after U-62066E or vehicle administration, after which, the hypertonic saline solution was infused. The rats were killed by decapitation just 10 min after the start of infusion and the blood was collected for determinations of pAVP and pOsm.

Plasma AVP was determined by the method previously described (Yamada *et al.*, 1989b). AVP in EDTA-treated plasma was measured by radioimmunoassay (RIA). In brief, after acidifying the plasma to pH 3.5 with 0.1 N HCl, a sample was passed through a column of octadecylsilyl silica (Seppak C18 cartridges) which had been prewashed with methanol (8 ml) and then with water (12 ml); the column was then washed with 4% acetic acid (10 ml) and AVP was eluted with 2.0 ml of methanol. The eluate was dried under nitrogen gas and the residue was reconstituted with RIA buffer. AVP was determined by radioimmunoassay. The lower limit of detection of the assay was 0.05 pg per tube (B/B_0 = 95%). B/B_0 of 50% was 0.85 pg per tube. The antibody used did not cross-react with oxytocin, bradykinin, α-human atrial natriuretic polypeptide, angiotensin I, or angiotensin II. Inhibitory potencies of these peptides were 1% or less, judged by their ability to achieve half maximal inhibition (B/B_0 = 50%). The recovery of AVP was 83.0 ± 2.1% (mean ± s.e.) when unlabelled AVP was added to plasma (0.5 to 8.0 pg ml^{-1}) before extraction. The intra-assay and interassay variabilities were 9.5 ± 0.6% and 10.6 ± 0.9%, respectively. In AVP assay with small sample volumes, the volume factor was checked by taking 0.2 ml, 0.3 ml, 0.5 ml or 1.0 ml of the pooled plasma sample (2.0 pg ml^{-1}) extracting and assaying AVP by the same procedure. Plasma AVP could be assayed linearly down to a sample volume of 0.2 ml. Sodium and potassium concentrations were determined by flame photometry. The osmolality of plasma was determined by freezing point depression.

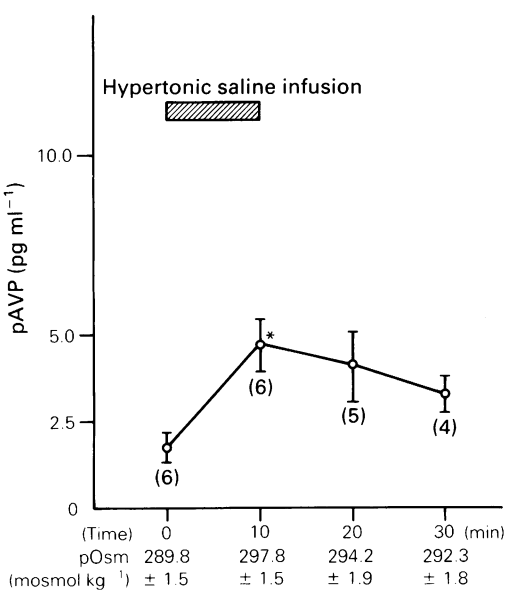

Figure 1 Time course of plasma arginine vasopressin (AVP) concentration changes in response to the infusion of hypertonic saline solution. Numerals in parentheses indicate the number of rats. Plasma osmolality is indicated below each time point. *$P < 0.01$.

Materials

[^{125}I]-AVP was purchased from New England Nuclear (Boston, MA, U.S.A.). Anti-AVP rabbit serum was donated by Mitsubishi Yuka (Tokyo, Japan). Seppak C18 cartridges were obtained from Waters Associates, Millipore (Milford, MA, U.S.A.). MR2266 ((−)-2-(3-furylmethyl)-noretazocine) was donated by Boehringer-Ingelheim (West Germany). AVP, bradykinin, angiotensin I, angiotensin II, α-human ANP and oxytocin were purchased from Protein Research Institute (Osaka). U-62066E ((5α,7α,8β)-(±)-3,4-dichloro-N-methyl-N-(7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl)benzeneacetamide methanesulphonate) was obtained from Upjohn Pharmaceuticals. Other reagents used were of analytical grade.

Statistical analysis

Data are expressed as mean ± s.e.mean. Statistical analysis was performed with the Student's paired or unpaired *t* test.

Results

Haemorrhage

The changes in blood pressure during the experiment are shown in Figure 2. No significant change in blood pressure or in plasma AVP levels (B_1) was observed in these conscious rats followed the administration of U-62066E or the vehicle, and/or MR2266, as compared to the control (Figure 2 and Table 1). In addition, no significant difference in mean blood pressure or in plasma AVP levels (B_1) just before the start of haemorrhage among these groups was observed (Figure 2 and Table 1).

Table 1 Effect of i.p. administration of U-62066E and/or MR2266 on plasma arginine vasopressin (AVP)

	Plasma AVP (pg ml^{-1})	
Drugs	B_0 (0 min)	B_1 (20 min)
Control (saline) (n = 6)	1.78 ± 0.38	1.09 ± 0.21
U-62066E (0.2 mg kg^{-1}) (n = 9)	2.11 ± 0.34	1.75 ± 0.37
U-62066E (2.0 mg kg^{-1}) (n = 12)	1.94 ± 0.35	1.47 ± 0.26
U-62066E (2.0 mg kg^{-1}) + MR2266 (0.2 mg kg^{-1}) (n = 6)	2.48 ± 0.41	1.44 ± 0.31
U-62066E (2.0 mg kg^{-1}) + MR2266 (2.0 mg kg^{-1}) (n = 6)	1.94 ± 0.62	1.60 ± 0.35
MR2266 (2.0 mg kg^{-1}) (n = 4)	1.61 ± 0.33	1.72 ± 0.38

Abbreviations: n: number of rats; B_0: plasma before drug administration; B_1: plasma at 20 min after U-62066E or saline administration.

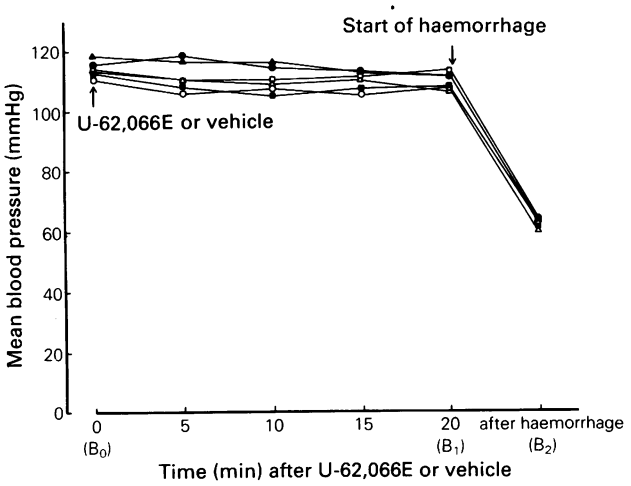

Figure 2 Effect of i.p. administration of U-62066E and/or MR2266, and haemorrhage on mean blood pressure in Long-Evans rats: (○) vehicle (saline) [number of rats (n) = 6]; (△) 2.0 mg kg^{-1} U-62066E (n = 9); (□) 2.0 mg kg^{-1} U-62066E (n = 12); (▲) 2.0 mg kg^{-1} U-62066E + 0.2 mg kg^{-1} MR2266 (n = 6); (●) 2.0 mg kg^{-1} U-62066E + 2.0 mg kg^{-1} MR2266 (n = 6); (■) vehicle (saline) + 2.0 mg kg^{-1} MR2266 (n = 4).

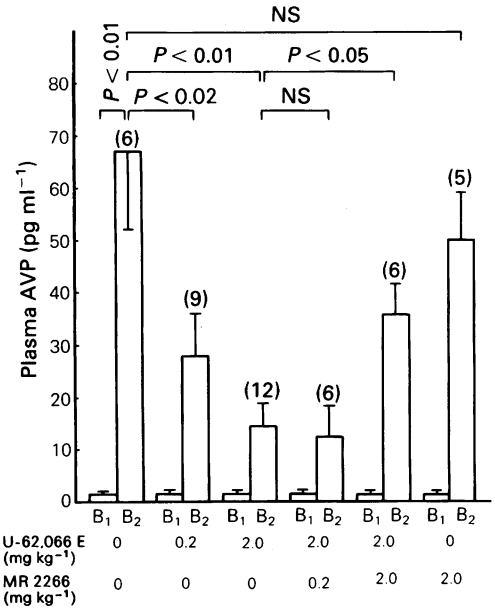

Figure 3 Effects of U-62066E and/or MR2266 on haemorrhage-induced plasma arginine vasopressin (AVP) concentrations. NS: not significant. Numerals in parentheses indicate the number of experiments. B_1: plasma just before haemorrhage; B_2: plasma after haemorrhage.

An acute haemorrhage reduced the mean blood pressure by approximately 50% without showing any difference in decrement among different drug-treated groups. Acute haemorrhage also resulted in a huge increase in the plasma AVP levels of control rats (Figure 3). However, the administration of U-62066E reduced the increase due to haemorrhage in a dose-dependent manner (Figure 3). In contrast, concomitant administration of 2.0 mg kg^{-1} of MR2266 with U-62066E partially prevented the inhibitory effect of 2.0 mg kg^{-1} of U-62066E on plasma AVP. On the other hand, the administration of 2.0 mg kg^{-1} of MR2266 alone did not significantly affect the increase in plasma AVP levels following haemorrhage.

In spite of the marked changes in the plasma AVP levels, plasma osmolality did not show any significant differences among the different groups, indicating that plasma osmolality was not a determinant for the changes in plasma AVP under these experimental conditions.

Hypertonic saline infusion

As shown in Figure 4, hypertonic saline infusion caused an elevation of plasma osmolality in the control rats, simultaneously resulting in a significant increase of pAVP levels. However, the administration of U-62066E reduced the osmotically induced increase in pAVP in a dose-dependent manner (Figure 4). At the same time, plasma osmolality

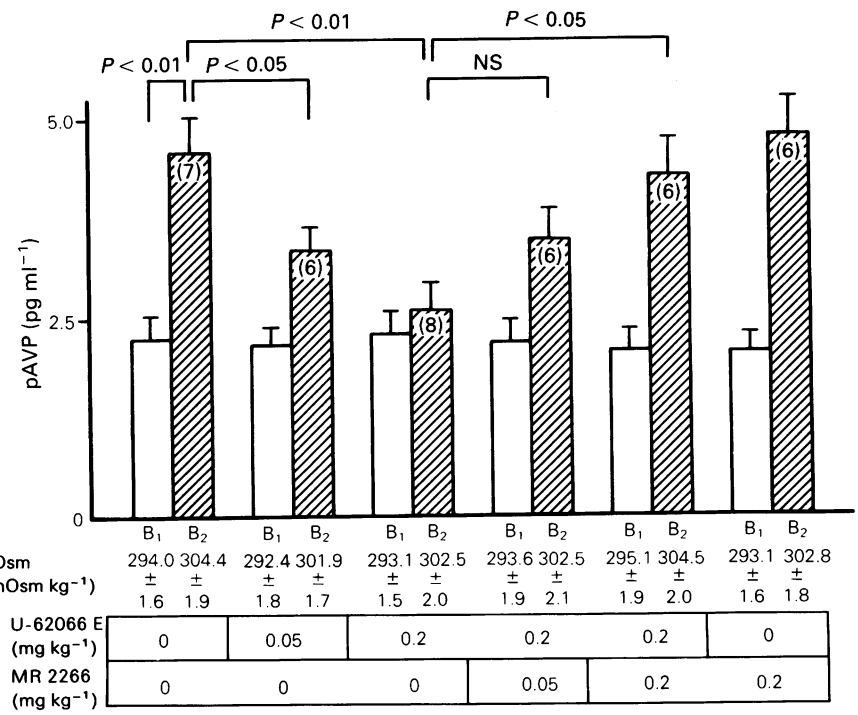

Figure 4 Effect of U-62066E and/or MR2266 on plasma arginine vasopressin (AVP) levels when hypertonic saline solution was infused into Long-Evans rats. NS: not significant. Numerals in parentheses indicate the number of experiments. B_1: plasma just before the infusion of hypertonic saline; B_2: plasma at 10 min after the start of the infusion. Plasma osmolality is indicated below each plasma sample.

showed the same degree of increase as that of control. In contrast, concomitant administration of MR2266 with U-62066E partially prevented the inhibitory effect of U-62066E, whilst showing the same degree of increase in plasma osmolality as that of control (Figure 4). No significant changes in the mean blood pressure of any group were observed during this experiment.

Discussion

The secretion of AVP is stimulated both by osmotic and by non-osmotic stimuli (Schrier *et al.*, 1979) such as hypotension, hypovolaemia and hypoglycaemia. Osmotic stimulation is mediated by hypothalamic osmoreceptors (Verney, 1947), which connect to the neurohypophysis through the supraoptic nucleus (SON) and paraventricular nucleus (PVN). On the other hand, non-osmotic, baroreceptor and volume-receptor mediated AVP responses appear to be mediated through many of the safe afferent pathways, including the vasomotor centre in the brain stem, that regulate sympathetic activity (Share, 1976; Schrier *et al.*, 1977).

Although the haemorrhage examined in the present study is thought to be a complex cardiovascular stimulus, it seems, at least in part, to cause AVP secretion by the activation of the baroreceptor-reflex arc (Ginsburg & Heller, 1953; Laycock *et al.*, 1979). Therefore, the impulses evoked by the two stimuli used (i.e. osmotic stimulus and acute haemorrhage) run along separate afferent pathways to the SON and PVN to stimulate AVP secretion. Thereafter a common pathway is involved (Zerbe *et al.*, 1983).

It is well known that neuropeptide secretion is modulated by opioids (Clarke *et al.*, 1979). The present study demonstrates that the administration of this highly selective κ-opioid receptor agonist, U-62066E, dose-dependently inhibited the acute AVP response to two quite different stimuli and that the inhibition was blocked by a κ-opioid receptor antagonist, MR2266. This strongly suggests that the κ-opioid receptor mechanism is mediated at the level of AVP secretion. κ-Opioid receptors have been found to be abundant in neurosecretosome preparations (Falke & Martin, 1985; Pesce *et al.*, 1987). An autoradiographic analysis has shown that pituitary κ-opioid receptor-binding was confined to the neural lobe where it was densest in the external rim (Herkenham *et al.*, 1986). Accordingly, it is suggested, from the present study, that the increase in the secretion of AVP with two different stimuli was inhibited somewhere during the final common pathway in the neural lobe, and that probably the activation of κ-opioid receptors might inhibit the release of AVP from nerve terminals and/or processes of pituicytes (Herkenham *et al.*, 1986).

Substantial amounts of dynorphin peptides (dynorphin A (1–17) and its related peptide dynorphin A (1–8)), which are thought to be endogenous κ-opioid receptor agonists (Chavkin & Goldstein, 1981), are found in the rat neurohypophysical system localized within AVP-containing magnocellular perikarya and terminals (Watson *et al.*, 1982). At a subcellular level they co-localized with AVP in common neurosecretory vesicles (Whitnall *et al.*, 1983). Furthermore, neurohypophysial dynorphin levels have been found to vary in parallel with AVP levels during physiological stimulation (Zamir *et al.*, 1985). Therefore, it might be speculated that endogenous κ-opioids, especially dynorphins, are involved in controlling the release of AVP from the pituitary in the final common pathway in the process of AVP secretion from an acute haemorrhage or hypertonic saline infusion.

The presence of κ-opioid binding activity in the rat hypothalamus, preoptic area, central nucleus of the amygdala, and rostral nucleus of the solitary tract, where they outnumber μ-receptors, has been reported (Herkenham *et al.*, 1986). Moreover, Carter & Lightman (1985) reported that a κ-opioid receptor agonist, U-50488H, markedly increased plasma AVP when it was microinjected into the nucleus tractus solitarius of rats. The reason for this discrepancy remains to be determined. Perhaps it may be due to the differences in experimental conditions. It may also be possible that the reported stimulation of AVP secretion is mediated by a separate mechanism occurring at the level of the nucleus tractus solitarius.

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