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κ-Opioid-receptor agonists modulate the renal excretion of water and electrolytes in anaesthetized rats

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1 Subcutaneous injection of the κ-opioid agonists U50,488 (10 mg kg^{-1}) and tifluadom (3.5 mg kg^{-1}) into Inactin-anaesthetized, saline-infused rats was associated with a diuresis, antinatriuresis and antikaliuresis which lasted for up to 2 h. A high (5 mg kg^{-1}), but not low (0.1 mg kg^{-1}), dose of naloxone blocked the renal effects of U50,488.

2 U50,488 administration in anaesthetized, vasopressin-deficient Brattleboro DI rats was associated with an attenuated diuresis, though the antinatriuretic response remained intact.

3 The diuretic action of U50,488 was associated with an increase in glomerular filtration rate while fractional fluid reabsorption remained steady. In contrast, fractional sodium and potassium reabsorption were increased.

4 These data suggest that κ-opioid agonists alter renal handling of both water and electrolytes. This appears to be mediated by two separate mechanisms: increased fluid loss largely reflects altered glomerular events while the fall in electrolyte excretion results from altered tubular handling.

Introduction

It is well established that κ-opioid agonists produce a profound diuresis in the rat (Slizgi & Ludens, 1982; Leander, 1983a, b; Von Voigtlander *et al.*, 1983), though the mechanisms involved are not clear. Miller (1975) attributed this effect to an inhibition of vasopressin (AVP) secretion, while Huidobro-Toro & Parada (1985) showed that bremazocine increased plasma AVP levels. We have recently demonstrated that the κ-receptor agonist U50,488 did not alter circulating AVP levels (Ashton *et al.*, 1989). κ-Agonists may alter urine flow via a direct renal action, such as a decrease in tubular reabsorption of water as reported by Slizgi *et al.* (1984), perhaps through a modulation of the action of AVP at the kidney.

In addition to the diuretic effects of U50,488, we have also observed an antinatriuresis in the conscious rat (Ashton *et al.*, 1989). Such an effect is not well documented and, although Slizgi *et al.* (1984) reported a similar retention of sodium in the anaesthetized dog, the renal mechanisms involved in this action of κ-agonists are unknown. Our previous study (Ashton *et al.*, 1989) demontrated that the diuretic and antinatriuretic actions of U50,488 may be mediated separately, since the diuretic response was attenuated in the AVP-deficient Brattleboro (DI) rat while the antinatriuretic response remained intact. In this study we have used an anaesthetized rat preparation in order to investigate further the renal actions of the κ-agonist U50,488. In particular we have used the inulin clearance technique to measure glomerular filtration rate (GFR) and to assess fractional tubular reabsorption of filtered water, sodium and potassium.

A preliminary account of some of these data was presented to the Society of Endocrinology (Balment *et al.*, 1986a).

Methods

Animals

Adult male Long Evans (420–480 g), Brattleboro rats homozygous for diabetes insipidus (DI 300–400 g) and Sprague-Dawley (SD 250–350 g) rats were obtained from colonies maintained at the University of Manchester. Male Alderley Park Wistar (APW 250–300 g) rats were obtained from colonies maintained at ICI Pharmaceuticals. A 12 h light:12 h dark photoperiod was maintained and animals had free access to food (Labsure PMD diet) and water. Animals were not fasted before experimentation.

Surgical procedure

Groups of Long Evans and Brattleboro DI rats were prepared as described in earlier studies (Forsling *et al.*, 1982). Rats were anaesthetized with Inactin (5-ethyl-5-(1'-methylpropyl)-2-thiobarbiturate 100 mg kg^{-1} i.p., Byk Gulden, West Germany) and placed on a continuous jugular infusion of 0.077 M NaCl at 150 μl min^{-1} (Sage Syringe pump model 351, MA, U.S.A.). The urinary bladder was cannulated (PP100, Portex, Kent), for the collection of urine into pre-weighed plastic vials. Body temperature was maintained at 37 ± 1°C by means of a heated table. Following a 3 h equilibration period, two × 20 min control urine collections were made before the animals received a s.c. injection of the test substance; 20 min urine collections were continued for a further 3 h. Urine volume, measured gravimetrically, and urinary sodium and potassium concentrations, measured by flame photometry (Corning model 455 flame photometer) were determined over the whole experimental period.

Long Evans rats prepared in this manner received s.c. injections of either 0.154 M NaCl (vehicle $n = 6$), U50,488 ($n = 5$), tifluadom ($n = 6$), low dose naloxone ($n = 5$), high dose naloxone ($n = 5$), U50,488 and low dose naloxone ($n = 6$) or U50,488 and high dose naloxone ($n = 5$). DI rats received s.c. injections of 0.154 M NaCl ($n = 6$) or U50,488 ($n = 6$).

Renal clearances

Glomerular filtration rate (GFR) was determined by the inulin clearance technique. Inactin-anaesthetized SD rats ($n = 18$) were prepared as described above, with the addition of a femoral artery cannula (PP50, Portex, Kent) to facilitate repeated blood sampling. Following 1 h of hypotonic saline infusion, the infusate was supplemented with [^{3}H]-inulin (0.044 μCi ml^{-1}, Amersham International Limited) for the remainder of the study. After the 3 h equilibration period, 20 min urine collections were made as before, and blood samples (0.4 ml) were taken from the femoral artery at 1 h intervals. An equivalent volume of hypotonic saline was

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slowly injected to replace the withdrawn blood. Following the two × 20 min control collections, animals received s.c. injections of either vehicle ($n = 9$) or U50,488 ($n = 9$).

[^{3}H]-inulin content of 100 μl of urine and 100 μl of plasma was determined at each time point using a Tri-Carb liquid scintillation system (10 min count, Packard Instrument Co. Inc.). Sodium and potassium concentration was determined in both plasma and urine samples. GFR (ml 100 g^{-1} b.wt. min^{-1}) was calculated from:

$$\text{GFR} = \frac{\text{urine volume}}{(\text{ml } 100\,\text{g}^{-1}\,\text{b.wt.min}^{-1})} \times \frac{\text{urine [inulin] (c.p.m. } 100\,\mu\text{l}^{-1})}{\text{plasma [inulin] (c.p.m. } 100\,\mu\text{l}^{-1})}$$

and fractional reabsorptions of fluid, sodium and potassium were determined as the differences between filtered load and urinary fluid and electrolyte loss.

Cardiovascular actions of U50,488

Under Saffan (12 mg kg^{-1} alphaxolone/alphadolone acetate, Glaxovet) anaesthesia, an exteriorised cannula (PP80, Portex, Kent) was inserted into the left carotid artery of adult male Alderley Park Wistar (APW) rats ($n = 16$). The cannula was flushed with heparinised saline to prevent blockage. Animals were kept in isolation and allowed at least 24 h to recover. Individuals were placed in open-ended restraining tubes and allowed a 0.5 h adaptation period. The cannula was connected to a pressure transducer (Gould Statham P231D), linked to a chart recorder (Devices MX19), to allow monitoring of mean arterial blood pressure (MAP) and heart rate (HR). Following stabilization of MAP and HR, U50,488 ($n = 8$) or vehicle ($n = 8$) was injected subcutaneously and MAP and HR were monitored for 120 min.

Long Evans rats ($n = 5$) anaesthetized with Inactin were prepared as described for the renal studies with the addition of a femoral artery cannula (PP50, Portex, Kent) to facilitate the direct measurement of mean arterial blood pressure. The cannula was connected to a pressure transducer (Gould Statham, U.S.A.) linked to a chart recorder (Grass Polygraph, U.S.A.) to allow continuous monitoring of MAP. Following 3 h of hypotonic saline infusion, animals received a subcutaneous injection of U50,488 (10 mg kg^{-1}) and MAP was monitored for the following 90 min.

Statistical analysis

All values are presented as the mean ± s.e.mean. Statistical comparisons were by Student's t test for paired and unpaired data, as appropriate. Where necessary, a modified t test for multiple group comparisons was also employed.

Drugs

The κ-opioid agonists U50,488 (*trans*-3,4,-dichloro-N-methyl-N[2-(1-pyrrolidinyl) cyclohexyl] benzeneacetamine methane sulphate) and tifluadom (both synthesized at ICI Pharmaceuticals, Macclesfield, U.K.) were administered, by subcutaneous (s.c.) injection, at a dose of 10 and 3.5 mg kg^{-1} b.wt. respectively. Naloxone (Sigma, Poole, Dorset) was administered, by s.c. injection, at a high (5 mg kg^{-1} b.wt.) and low (0.1 mg kg^{-1} b.wt.) dose. Control animals received vehicle only injections (0.154 M NaCl, 1 ml kg^{-1} b.wt.).

Results

Time course of responses to κ-receptor agonists

Administration of U50,488 was associated with a diuresis which reached a peak at 60 min after injection (Figure 1). Thereafter the urine flow rate declined, falling below control levels by two hours post-injection. This contrasts with the vehicle-injected group which maintained a stable urine flow rate over the study period (142.2 ± 3.3 μl min^{-1} cf. infusion rate of 150 μl min^{-1}). Tifluadom administration was associated with a similar diuresis, which was maximal 80 min after injection (control 136.2 ± 6.3 vs tifluadom 248.5 ± 9.2 μl min^{-1}, $P < 0.001$).

The diuretic actions of U50,488 and tifluadom were also associated with a marked antinatriuresis (U50,488 Figure 1; tifluadom control 7.1 ± 0.9 vs tifluadom 3.4 ± 0.6 μmol min^{-1}, $P < 0.001$) which was maximal at 100 and 40 min postinjection respectively. An antikaliuresis was also observed in both U50,488 (control 5.5 ± 0.2 vs U50,488 2.7 ± 0.4 μmol min^{-1}, $P < 0.001$) and tifluadom (control 4.4 ± 0.3 vs tifluadom 2.1 ± 0.5 μmol min^{-1}, $P < 0.001$)-treated animals, which was maximal at 60 min post-administration in both groups. Potassium, but not sodium, excretion had returned to rates comparable with control pre-injection levels by the end of the study. Vehicle-injected animals showed a progressive increase in sodium excretion over the study period (Figure 1), approaching the infusion rate of 11.55 μmol min^{-1} during the final hour of infusion. Potassium excretion, however, gradually declined, reflecting the absence of this cation from the infusate.

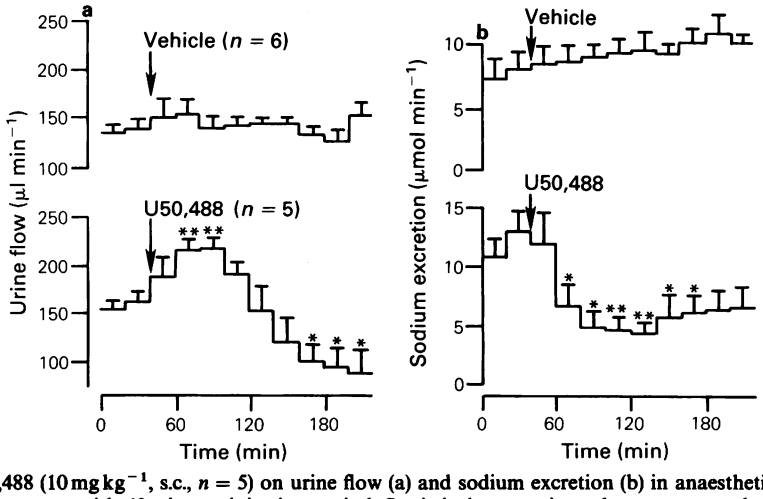

Figure 1 Effect of U50,488 (10 mg kg^{-1}, s.c., $n = 5$) on urine flow (a) and sodium excretion (b) in anaesthetized, saline-infused Long Evans rats. All comparisons are with 40 min pre-injection period. Statistical comparisons between control, pre-injection and experimental, post-injection measures are indicated by *$P < 0.05$; **$P < 0.01$.

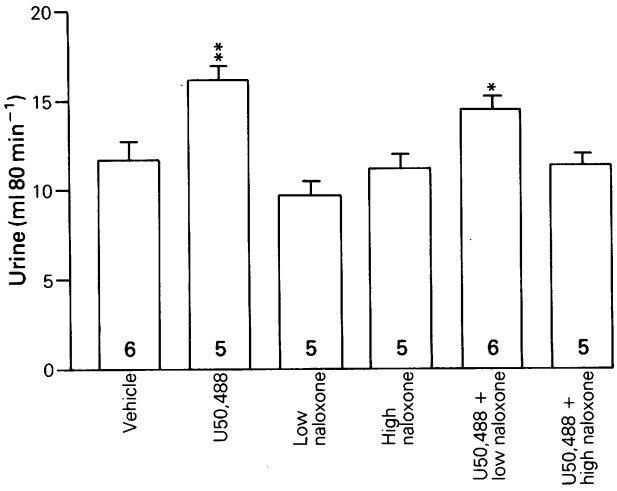

Figure 2 Reversal by a high dose of naloxone (5 mg kg^{-1}, s.c., $n = 5$), but not by a low dose of naloxone (0.1 mg kg^{-1}, s.c., $n = 5$) of the effect of U50,488 (10 mg kg^{-1}, s.c., $n = 6$) on cumulative urine output over 80 min post-injection in anaesthetized, saline-infused Long Evans rats. Statistical comparisons between vehicle and all other treated animals are indicated by $*P < 0.05$; $**P < 0.01$.

Naloxone inhibition of κ-receptor-mediated effects

κ-Receptor-mediated effects on renal water handling appeared to be maximal over the initial 80 min post-injection (Figure 1). The data presented in Figure 2 represent the mean urine flow rates over this 80 min period in animals receiving U50,488 and/or naloxone, an opioid receptor antagonist. Injection of U50,488 alone resulted in a significant increase in urine flow over the 80 min post-injection period compared with vehicle-injected animals. This effect was not altered by co-administration of a low dose of naloxone (0.1 mg kg^{-1}), which might be expected to block μ-receptor activity but the diuretic activity was attenuated by the higher dose of naloxone (5 mg kg^{-1}), which might be expected to block κ-receptor activity (Paterson *et al.*, 1983). Neither dose of naloxone alone altered urine flow rates.

A similar inhibition of the antinatriuretic activity of U50,488 was achieved by co-administration of a high dose of naloxone. The maximal antinatriuresis induced by U50,488 at 100 min post-injection (Figure 1 control 9.9 ± 1.3 vs U50,488 4.4 ± 0.9 μmol min^{-1}, $P < 0.01$) was not inhibited by co-administration of a low dose of naloxone (5.6 ± 1.6 μmol min^{-1}), but was blocked by the higher dose of naloxone (9.2 ± 1.5 μmol min^{-1}, $P < 0.05$ vs U50,488). Again, both low (7.9 ± 1.5 μmol min^{-1}) and high (10.1 ± 1.2 μmol min^{-1}) doses of naloxone alone do not significantly alter the rate of sodium excretion from that observed in the vehicle-treated group.

Absence of arginine-vasopressin

The diuretic response of vasopressin-replete rats was absent in the vasopressin-deficient Brattleboro DI rat. Indeed, urine flow rates were lower than control pre-injection levels 40 min after administration of U50,488 (control 207.3 ± 6.7 vs 40 min post-injection 176.1 ± 7.7 μl min^{-1}, $P < 0.05$). The antinatriuretic response to U50,488, however, was still apparent in DI rats, reaching a maximum 40 min after injection (control 5.99 ± 1.45 vs U50,488 2.04 ± 0.79 μmol min^{-1}, $P < 0.05$).

Renal clearances

The diuretic action of U50,488 was associated with a concurrent increase in glomerular filtration rate (GFR, control 0.40 ± 0.05 vs 80 min post-injection 0.516 ± 0.08 ml 100 g^{-1} b.wt. min^{-1}, $P < 0.05$). Throughout this period, fractional fluid reabsorption remained unaltered (Figure 3). This clearly suggests that the diuresis observed was due to altered glomerular rather than tubular events. The profound reduction in sodium and potassium excretion following

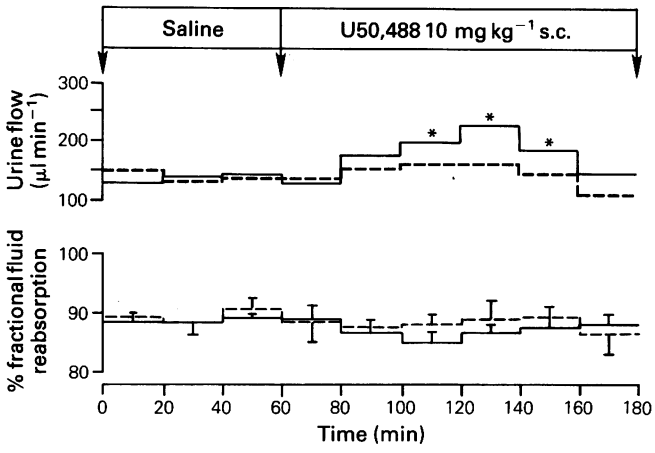

Figure 3 Effect of U50,488 (10 mg kg^{-1}, s.c., $n = 9$) on urine flow and fractional fluid reabsorption in anaesthetized, saline-infused SD rats. Continuous line, U50,488; broken line, vehicle. Statistical comparisons between control, pre-injection and experimental post-injection measures are indicated by $*P < 0.05$.

U50,488 seen in previous studies (Figure 1), was again evident in the clearance preparation, though sodium excretion rates were generally less stable. Forty min after U50,488 injection, sodium excretion was significantly lower than that observed in control, vehicle injected rats (control $n = 8$, 2.97 ± 0.36 vs U50,488 $n = 8$, 1.75 ± 0.41 μmol min^{-1}, $P < 0.05$). The fall in electrolyte excretion was associated with increased fractional sodium and potassium excretion (Figure 4). This suggests that the antinatriuresis and antikaliuresis associated with U50,488

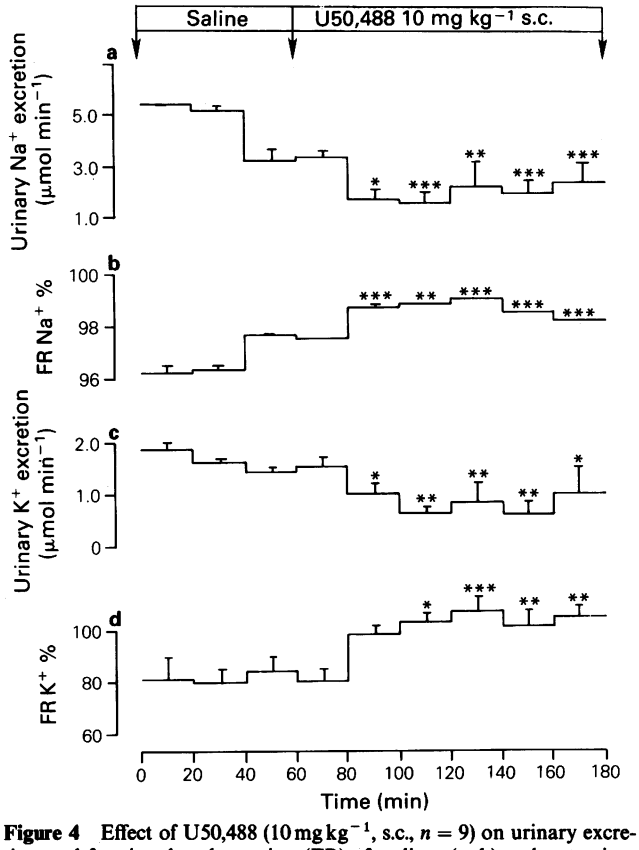

Figure 4 Effect of U50,488 (10 mg kg^{-1}, s.c., $n = 9$) on urinary excretion and fractional reabsorption (FR) of sodium (a, b) and potassium (c, d) in anaesthetised, saline-infused SD rats. Statistical comparisons between control, pre-injection and experimental post-injection measures are indicated by $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

administration, which occur in the face of an increased filtered load, were due to marked changes in tubular function.

Cardiovascular actions of U50,488

Administration of U50,488 by s.c. injection to conscious APW rats in comparison with vehicle-treated rats had no effect on either mean arterial blood pressure (116.8 ± 11.4 vs 120.5 ± 17.6 mmHg) or heart rate (431.8 ± 19.5 vs 414.2 ± 41.6 beats per min) over the 2 h period of observation.

In contrast, U50,488 administration in Inactin-anaesthetized Long Evans rats resulted in a fall in MAP (control 94.0 ± 5.6 mmHg, $n = 5$, mean fall in MAP over 60 min was 18.8 ± 1.4 mmHg, $P < 0.01$), which became apparent 3–4 min after s.c. injection of U50,488 and showed no sign of recovery within the next 90 min.

Discussion

The anaesthetized renal preparation employed in this study has enabled the detailed description of κ-receptor agonist actions on renal water and electrolyte handling. This model has prevously been used to investigate successfuly changes in renal function in response to a variety of endocrine manipulations (Forsling *et al.*, 1982; Balment *et al.*, 1986b; Ashton & Balment, 1988). Infusion of hypotonic saline produced a steady state diuresis without imposing a major sodium load, thus allowing subtle changes in renal function to be detected. U50,488 administration in this model was associated with changes in both fluid and electrolyte excretion rates that were comparable with our previously reported observations in conscious, saline stomach loaded LE and APW rats (Ashton *et al.*, 1989). Indeed, this anaesthetized preparation gave, perhaps, a clearer and more detailed indication of the renal actions of κ-receptor agonists than the conscious rat model.

In the conscious animal U50,488 injection resulted in a diuresis and an antinatriuresis which were apparent over a 3 h period, compared with the more rapid responses observed in the anaesthetized rat. However, the conscious animals were not in a steady state diuresis prior to administration of U50,488, which may account, in part, for the apparently extended period of action. In the anaesthetized animals a steady state diuresis was established before administration of the κ-agonist and thus the more rapid changes observed in renal function may reflect more accurately the activity of κ-agonists.

Inhibition of both the diuretic and antinatriuretic actions of U50,488 by co-administration of a high dose of naloxone suggests that both these effects are mediated through the κ-receptor subtype (Kosterlitz *et al.*, 1981; Wood *et al.*, 1981; Leander, 1983b). This is supported by the observation that a low dose of naloxone, which might be expected to block only μ-receptor-mediated activity, did not inhibit the renal actions of U50,488. However, whilst U50,488 exerts its effects on renal water and electrolyte handling through the κ-receptor, it appears that it achieves these actions via two separate mechanisms.

This concept is supported by the changes in GFR and fractional sodium excretion and the absence of change in fracional fluid reabsorption observed upon U50,488 administration. Slizgi *et al.* (1984) suggested that the diuretic effects of U50,488 may reflect a decrease in tubular reabsorption of water, since GFR was not altered by doses of U50,488 up to $5\,mg\,kg^{-1}$ i.v. (cf. $10\,mg\,kg^{-1}$ s.c. in this study) in the anaesthetized dog. Our data, however, show that fractional fluid reabsorption remained unaltered and that the diuresis was apparently due to an increase in GFR. Such an increase in GFR and associated increase in filtered load might also be expected to cause a natriuresis, however U50,488 administration resulted in an antinatriuresis which was attributable to a marked increase in fractional sodium reabsorption. Unusually, a similar increase in fractional potassium reabsorption was also observed. These data suggest that U50,488 has separate actions at different parts of the nephron.

It is not clear from these data how U50,488 affects GFR. The dose and route of administration employed in this study in anaesthetized rats resulted in a fall in systemic blood pressure of approximately 20 mmHg, but it is not known if this was reflected in any local change in renal blood flow. Slizgi *et al.* (1984) reported that renal plasma flow was unaltered by i.v. (cf. s.c.) injections of U50,488 up to $5\,mg\,kg^{-1}$ in the anaesthetized dog. However, they also reported a fall in mean arterial blood pressure in excess of 40 mmHg at this dose, which contrasts with our observations in the rat. This may reflect effects of the different routes of administration of the κ-agonist. Clark *et al.* (1988) found that U50,488 administered i.v. to urethane-anaesthetized rats resulted in large fall in blood pressure, 27 mmHg at $0.79\,mg\,kg^{-1}$ and 36 mmHg at $7.9\,mg\,kg^{-1}$. It seems unlikely that the depressor activity of U50,488 could have resulted in an increase in GFR directly, however, compensatory mechanisms, particularly the renin-angiotensin system, acting to maintain blood pressure may contribute to the enhanced rate of glomerular filtration. U50,488 administration was associated with an increase in plasma renin activity in the conscious animal (Ashton *et al.*, 1989), hence the increase in GFR may be mediated through an increase in angiotensin II levels (Blantz & Pelayo, 1983; Ichikawa & Brenner, 1984). Similarly, plasma corticosterone levels rose in the conscious animal, which may also act to increase GFR (Goldsmith *et al.*, 1962). The potential role of AVP in these events is less clear. The diuretic actions of κ-agonists were initially attributed to an inhibition of AVP secretion (Miller, 1975), however we have recently shown that plasma AVP levels did not alter in response to U50,488 administration (Ashton *et al.*, 1989) and Huidobro-Toro & Parada (1985) have reported that bremazocine increased circulating AVP, which might be expected to decrease GFR (Schor *et al.*, 1981). The picture is complicated by the observation that the κ-agonist induced diuresis is absent in the AVP-deficient Brattleboro DI rat, suggesting that AVP may be involved in the action of κ-agonists. The nature of this involvement remains obscure, although it appears that the pressor activity of AVP is not involved, since pretreatment with a V_1 antagonist, desglycinamide deamino-[Arg^8]-vasopressin did not affect a tifluadom-induced diuresis (Blackburn *et al.*, 1986).

The mechanisms involved in the U50,488-mediated increase in fractional sodium and potassium reabsorption are also unclear. Previous studies in conscious animals by other workers have not detected this retention of electrolytes as urine collection periods were too long (5 h) (e.g. Slizgi & Ludens, 1982; Von Voigtlander *et al.*, 1983; Huidobro-Toro & Parada, 1985), so there are few data available for comparison with our study. Slizgi *et al.* (1984) reported an antinatriuresis in the anaesthetized dog, but did not give an indication of fractional sodium reabsorption. Aldosterone does not appear to mediate this response to U50,488, since plasma aldosterone levels in the conscious rat were not altered (Ashton *et al.*, 1989); however, corticosterone levels have been shown to rise in several studies, possibly as a result of the stress response evoked by κ-opiates (Lahti & Collins, 1982; Von Voigtlander *et al.*, 1983; Eisenberg, 1985; Iyengar *et al.*, 1986; Ashton *et al.*, 1989). U50,488 may have a direct action on both sodium and potassium reabsorption, since renal κ-opioid binding sites have been identified (Quirion *et al.*, 1983). This may account for the increased reabsorption of both cations, particularly against the background of increased tubular flow. It is not easy to envisage a mechanism whereby direct hormonal action could result in retention of both sodium and potassium under these conditions. Clearly, further investigation is required to clarify this point.

In conclusion, we have shown that the diuretic, antinatriuretic and anti-kaliuretic actions of the κ-agonist U50,488 demonstrated in the conscious animal are apparent

in our anaesthetized preparation. The U50,488-induced diuresis was apparently attributable to an increase in GFR, while the retention of sodium and potassium was due to an increase in fractional reabsorption of these cations. The concept that the renal actions of κ-agonists are mediated by separate mechanisms is supported by the observation that the diuretic response was absent in AVP-deficient, Brattleboro DI rats, while the antinatriuretic response remained.

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