κ -Opioid-induced changes in renal water and electrolyte management and endocrine secretion

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1 Subcutaneous injection of the κ -opioid agonist U50,488 into conscious, saline-loaded rats was associated with a diuresis, antinatriuresis and antikaliuresis which lasted for up to 3 h. Plasma renin activity and corticosterone levels were elevated but plasma vasopressin (AVP) and aldosterone levels were unaltered in similarly treated rats.

2 U50,488 administration to adrenal demedullated rats was not associated with a diuresis but produced an antinatriuresis, though sodium excretion rates were higher in demedullated than in sham-operated animals. Plasma AVP and corticosterone levels were not affected by demedullation or subsequent U50,488 treatment. Sham-operated, U50,488-treated rats showed the expected increase in plasma corticosterone levels.

3 U50,488 administration resulted in an antidiuresis and an antinatriuresis in AVP-deficient Brattleboro DI rats.

4 When coupled with fasting stress U50,488 administration resulted in similar but attenuated renal responses compared with those observed in unfasted rats. Basal plasma corticosterone levels were elevated in fasted animals and were further increased by U50,488.

5 Both water and electrolyte handling by the kidney are altered by U50,488. The diuretic effects of U50,488 were reversed by adrenal demedullation and in the absence of endogenous AVP, but the antinatriuretic actions were not altered, suggesting that the effects upon renal water and electrolyte excretion may be mediated by separate mechanisms.

Introduction

κ -Opioid agonists have been shown to produce a profound diuresis in the rat (Slizgi & Ludens, 1982; Leander, 1983a,b; VonVoigtlander *et al.*, 1983) which was initially attributed to inhibition of vasopressin (AVP) secretion (Miller, 1975). More recently, however, the κ -agonist bremazocine has been shown to increase circulating plasma AVP levels yet still cause a diuresis (Huidobro-Toro & Parada, 1985), suggesting that other factors acting in the periphery may be involved. Removal of the adrenal medulla has been shown to attenuate the diuretic actions of the κ -agonist U50,488 (Blackburn *et al.*, 1986), although the reasons for this effect were not clear. Adrenal medullary AVP (Ang & Jenkins, 1984) did

not appear to exert a direct influence on renal water handling, since untreated demedullated rats maintained stable water and salt balances (Blackburn *et al.*, 1986), suggesting that some other medullary factor may interact with κ -opioids.

Although the diuretic response to κ -agonists has been well documented, potential effects on renal electrolyte handling have not been fully investigated. Previously described protocols involving long (5 h), single sample urine collections may have masked short term responses. Accordingly, we have used a more frequent sampling protocol in order to investigate the time course of effects of U50,488 on renal water and electrolyte handling. In addition, the influence of the adrenal medulla in κ -opioid-induced diuresis and the role of AVP and other hormones have been further examined. A preliminary account of some of these data was presented to the Physiological Society (Ashton *et al.*, 1987).

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Methods

Animals

Adult male Alderley Park Wistar (APW, 180–210 g) and Brattleboro rats homozygous for diabetes insipidus (DI, 220–320 g) were obtained from colonies maintained at ICI Pharmaceuticals and the University of Manchester, respectively. A 12 h light:12 h dark photoperiod was maintained and animals had free access to food (Labsure PMD diet) and water. APW rats were routinely fasted overnight before experimentation.

Time course responses to U50,488

Pairs of APW rats ($n = 16$ pairs) were placed in metabolism cages (Jencons, Hertfordshire), 1 h before stomach loading with saline (0.154 M NaCl at 4 ml 100 g^{-1} , orally). Immediately following the stomach load, half of the animals were treated with U50,488, and the remaining animals received vehicle alone. Urine was collected at hourly intervals for 6 h. Sodium and potassium concentrations were measured by flame photometry (Model IL-943, International Laboratory apparatus, Warrington) and urine osmolality by freezing point depression (Camlab, Roebing Osmometer, Cambridge).

A second group of APW rats ($n = 47$) were similarly prepared and were killed by decapitation at 0.5 ($n = 15$), 1 ($n = 16$) or 2 h ($n = 16$) after administration of the agonist or vehicle. The separated plasma from collected trunk blood was stored at -20°C before radioimmunoassay for arginine vasopressin and corticosterone concentrations. Plasma samples obtained from animals killed 2 h after injection were also subjected to radioimmunoassay for plasma renin activity and aldosterone.

Adrenal demedullation

Bilateral adrenal demedullation and sham operations were performed on groups of APW rats ($n = 16$ in each group) under Saffan (12 mg kg^{-1} i.v. Alphaxalone/Alphadolone acetate, Glaxovet) anaesthesia, according to the method described by Borkowski & Quinn (1983). All animals were used not less than seven days after surgery. Demedullated ($n = 8$ pairs) and sham-operated animals ($n = 8$ pairs) were housed in pairs in metabolism cages and received a saline stomach load, as described above. Animals received injections of either U50,488 or vehicle alone and urine was collected for 0.5, 1 or 2 h. Analyses of sodium and potassium content and osmolality were as described above.

Further groups of demedullated ($n = 46$) and sham-operated ($n = 40$) animals were prepared in a

similar manner and were killed by decapitation at either 0.5, 1 or 2 h after injection of U50,488 or vehicle. The separated plasma was stored at -20°C prior to radioimmunoassay for arginine vasopressin and corticosterone concentrations.

Absence of arginine vasopressin

Groups of DI rats were housed individually in metabolism cages before saline loading (0.154 M NaCl, 2.5 ml 100 g^{-1} orally) and s.c. injection of U50,488 ($n = 7$) or vehicle ($n = 8$). Urine was collected over the following 2 h. Sodium and potassium concentrations were determined by flame photometry (Corning 455 flame photometer) and osmolality by freezing point depression (Roebing Automatik Osmometer, L.H. Roebing, Berlin, W. Germany).

Effects of fasting

Groups of APW rats were either fasted overnight ($n = 4$ pairs) as usual or allowed free access to food ($n = 4$ pairs) before the normal stomach loading procedure and administration of U50,488 or vehicle, as described above. Urine was collected over a 2 h period and sodium and potassium concentrations and osmolality were determined. Animals were killed by decapitation 2 h after injection and the separated plasma was stored at -20°C prior to radioimmunoassay for corticosterone concentration.

Radioimmunoassays

Plasma arginine vasopressin was measured by specific radioimmunoassay following extraction with acetone and petroleum ether using the method described by Ashton & Balment (1988). Inter- and intra-assay coefficients of variation were 14.6% ($n = 12$) and 17.1% ($n = 10$) respectively. The minimum detectable plasma vasopressin concentration was $0.1\text{ }\mu\text{g ml}^{-1}$ ($1\text{ }\mu\text{g} = 2.2\text{ pg}$).

Plasma corticosterone was measured in ethanol extracted samples according to the method of Kime (1977). Inter- and intra-assay coefficients of variation were 10.1% ($n = 30$) and 13.6% ($n = 10$) respectively. The minimum detectable plasma corticosterone concentration was $400\text{ pg }100\text{ ml}^{-1}$.

Plasma aldosterone was measured following initial separation from other steroids by LH20 chromatography by the method described by Milne *et al.* (1982). Inter- and intra-assay coefficients of variation were 12.7% ($n = 42$) and 14.4% ($n = 10$) respectively. The minimum detectable plasma aldosterone concentration was $2\text{ pg }100\text{ ml}^{-1}$.

Figure 1 Effect of U50,488 (10 mg kg^{-1} s.c., $n = 8$ pairs, solid line) and vehicle administration (0.154 M NaCl , $n = 8$ pairs, dashed line) on urine output (a), sodium (b) and potassium excretion (c) and urine osmolality (d) in conscious saline loaded ($4 \text{ ml } 100 \text{ g}^{-1}$, orally) APW rats. Statistical comparisons between vehicle- and U50,488-treated groups are indicated by * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Plasma renin activity, expressed as the rate of angiotensin I generation, was measured by the method of Oldham *et al.* (1984).

Drugs

The κ -opioid agonist U50,488 (*trans*-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methane sulphate, synthesized at ICI Pharmaceuticals, Macclesfield) was administered, by subcutaneous injection, at a dose rate of 10 mg kg^{-1} . Control animals received vehicle only injections (0.154 M NaCl) at a rate of 1 ml kg^{-1} .

Statistical analysis

All values are presented as the mean $\pm$ s.e.mean. Statistical comparisons were by Student's *t* test for unpaired data.

Results

Time course responses to U50,488

κ -Agonist administration, at the submaximal dose of 10 mg kg^{-1} (Blackburn *et al.*, 1986), was associated with a diuresis which reached a peak at 2 h post injection (Figure 1). Urine output had fallen to levels comparable with those of the control, vehicle-injected animals by the fourth hour and gradually declined thereafter. Cumulative urine output over the 6 h study period was significantly higher in agonist-treated animals than in vehicle-injected rats (vehicle, 17.1 ± 2.6 vs U50,488, $38.3 \pm 3.9 \text{ ml } 6 \text{ h}^{-1}$, $P < 0.001$). Sodium and potassium excretion rates (Figure 1) were lower in U50,488- than in vehicle-treated rats over the first 3 and 2 h post injection respectively. This retention of sodium and potassium

Table 1 Plasma vasopressin (AVP) and corticosterone concentrations in intact, sham-operated and adrenal-demodulated conscious, saline-loaded (4 ml 100 g⁻¹, orally) APW rats killed at 0.5, 1 or 2 h after administration of U50,488 (10 mg kg⁻¹ s.c., *n* = 64) or vehicle (0.154 M NaCl, *n* = 69)

Plasma AVP ($\mu\text{u ml}^{-1}$)								
Time (h)	Intact		Sham		Demodulated		Vehicle	U50,488
	Vehicle	U50,488	Vehicle	U50,488	Vehicle	U50,488		
0.5	1.9 $\pm$ 0.5	2.7 $\pm$ 0.5	3.4 $\pm$ 0.5	2.9 $\pm$ 0.4	3.3 $\pm$ 0.4	3.9 $\pm$ 0.9		
1	1.3 $\pm$ 0.2	1.8 $\pm$ 0.5	1.6 $\pm$ 0.4	1.7 $\pm$ 0.7	2.7 $\pm$ 0.8	2.2 $\pm$ 0.7		
2	3.2 $\pm$ 0.5	2.8 $\pm$ 0.3	2.5 $\pm$ 0.6	2.7 $\pm$ 0.3	3.0 $\pm$ 0.6	3.9 $\pm$ 0.6		
Plasma corticosterone ($\mu\text{g } 100 \text{ ml}^{-1}$)								
Time (h)	Intact		Sham		Demodulated		Vehicle	U50,488
	Vehicle	U50,488	Vehicle	U50,488	Vehicle	U50,488		
0.5	9.9 $\pm$ 1.5	* 13.0 $\pm$ 0.4	9.7 $\pm$ 1.1	*** 14.7 $\pm$ 0.8	10.8 $\pm$ 0.8	10.6 $\pm$ 0.8		
1	6.2 $\pm$ 1.1	*** 13.4 $\pm$ 0.7	5.7 $\pm$ 1.0	** 10.7 $\pm$ 0.7	5.4 $\pm$ 0.6	7.1 $\pm$ 1.0		
2	10.5 $\pm$ 1.1	* 14.3 $\pm$ 0.9	8.4 $\pm$ 1.3	*** 16.7 $\pm$ 0.8	8.6 $\pm$ 0.9	10.1 $\pm$ 0.7		

Statistical comparisons between vehicle and U50,488-treated animals in each of the three groups are indicated by * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

was associated with a subsequent rebound natriuresis and kaliuresis over the final 2–3 h of the experimental period, such that cumulative excretion rates over 6 h did not differ between U50,488- and vehicle-injected animals (Na⁺ vehicle, 2130.0 $\pm$ 270.0 vs. U50,488, 2124.0 $\pm$ 306.0 $\mu\text{mol } 6 \text{ h}^{-1}$ NS; K⁺ vehicle, 624.0 $\pm$ 114.0 vs. U50,488, 756.0 $\pm$ 102.0 $\mu\text{mol } 6 \text{ h}^{-1}$, NS). In accordance with the retention of electrolytes and the diuresis shown by U50,488-treated rats, urine osmolality (Figure 1) was lower than that seen in control animals over the initial 3 h, and subsequently increased during the last 3 h of observation.

Over the first 2 h following κ -agonist injection, when the diuretic effects were maximal, plasma AVP levels (Table 1) did not differ from those measured in control animals. However, U50,488-treated rats had elevated plasma corticosterone (Table 1) but not aldosterone concentrations (2 h post-injection control, 156.6 $\pm$ 23.7 vs. U50,488, 153.6 $\pm$ 23.3 ng 100 ml⁻¹, NS). Plasma renin activity was also elevated 2 h after U50,488 injection compared with vehicle injected controls (control, 2.86 $\pm$ 0.59 vs. U50,488, 4.96 $\pm$ 0.40 ng angiotensin I ml⁻¹ h⁻¹, $P < 0.05$).

Adrenal demedullation

Adrenal demedullation attenuated the renal effects of U50,488. κ -Agonist administration in demedullated animals was associated with a much lower rate of urine production than in agonist-treated, sham-operated rats (Figure 2) or vehicle injected, demedullated rats (2 h, $P < 0.001$). Cumulative sodium excretion was elevated in U50,488-treated, demedullated animals compared with the similarly treated

sham-operated group (Figure 2), though this remained lower than that shown by vehicle-injected, demedullated animals (2 h, $P < 0.05$). Prior adrenal demedullation did not influence the antidiuretic action of U50,488 previously observed in intact animals. Cumulative potassium loss was reduced in U50,488-treated, demedullated animals compared with vehicle-injected, demedullated rats by 2 h (vehicle, 408.0 $\pm$ 24.0 vs. U50,488, 252.0 $\pm$ 24.0 μmol , $P < 0.001$). In accordance with these effects on water and electrolyte excretion, urine osmolality was higher following agonist treatment in demedullated animals than in the sham groups (2 h sham, 389.0 $\pm$ 111.0 vs. demedullated, 783.0 $\pm$ 36.0 mosm kg⁻¹, $P < 0.01$). However, there were no significant differences between U50,488 and vehicle-injected, demedullated animals.

Plasma AVP levels were not altered by adrenal demedullation nor U50,488 injection (Table 1). Plasma corticosterone, however, was significantly increased ($P < 0.01$) in U50,488-treated, sham-operated animals by comparison with the level in the other three treatment groups at each time point (Table 1). This elevation in plasma corticosterone was in accordance with the increase in corticosterone noted in intact rats in response to U50,488 (Table 1) and was notably absent following U50,488 administration in demedullated animals.

Absence of arginine vasopressin

The effects of U50,488 on renal water handling were altered in rats congenitally lacking AVP. In contrast to the diuretic response shown by AVP-replete animals (Figure 1) over the first 2 h following agonist administration there was a reduction in urine output

Figure 2 Effect of adrenal demedullation (stippled bars) and sham-operation (solid bars) on changes in urine output (a) and sodium excretion (b) in response to U50,488 (10 mg kg^{-1} s.c., $n = 8$ pairs) and vehicle administration (0.154 M NaCl , $n = 8$ pairs) in conscious, saline-loaded ($4 \text{ ml } 100 \text{ g}^{-1}$, orally) APW rats. Statistical comparisons between sham and demedullated animals receiving either U50,488 or vehicle are indicated by * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

in DI rats compared with vehicle-injected animals (Table 2). However, the retention of sodium and potassium observed in AVP-replete animals (Figure 1) upon U50,488 injection was also observed in AVP-deficient DI rats (Table 2). This retention of electrolytes in DI rats followed a similar time course to that observed in APW rats (Figure 1).

Table 2 Effect of U50,488 (10 mg kg^{-1} s.c., $n = 7$) and vehicle administration (0.154 M NaCl , $n = 8$) on the initial 2 h urine output and sodium and potassium excretion in conscious, saline-loaded ($2.5 \text{ ml } 100 \text{ g}^{-1}$, orally) vasopressin-deficient Brattleboro DI rats

	Vehicle		U50,488
Urine output (ml)	15.1 ± 2.1	**	6.5 ± 1.2
Sodium excretion (μmol)	323.0 ± 50.9	***	61.4 ± 24.1
Potassium excretion (μmol)	101.0 ± 14.8	*	57.3 ± 10.1

Statistical comparisons between vehicle and U50,488 treated animals are indicated by * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Effects of fasting

Overnight fasting before U50,488 administration was associated with similar, but attenuated, renal responses to the agonist compared with those shown by unfasted animals. Urine output was lower in fasted animals receiving either the vehicle or U50,488 compared with similarly treated, unfasted rats (Figure 3). Sodium excretion was also lower in vehicle-injected, fasted rats than in unfasted rats (Figure 3). Potassium excretion was reduced in fasted rats compared with unfasted rats in both vehicle (105.0 ± 19.9 vs. $547.8 \pm 147.7 \mu\text{mol}$, $P < 0.05$) and U50,488 (54.0 ± 6.9 vs. $261.0 \pm 53.2 \mu\text{mol}$, $P < 0.01$) treated groups. In contrast, plasma corticosterone concentrations were greater in both groups of fasted animals than in unfasted rats (Figure 3). A further increase in corticosterone associated with U50,488 injection (Table 1) was however, still observed in the fasted animals (Figure 3).

Discussion

It has been proposed that the diuretic effect of κ -agonists is due entirely to a suppression of vasopressin release, since this effect is absent in Brattleboro rats which are homozygous for diabetes insipidus (Leander, 1983b), and is abolished by administration of the synthetic vasopressin analogue, desmopressin (Leander *et al.*, 1985). However, in the present studies no changes were apparent in vasopressin levels in normal, sham-operated or demedullated saline-loaded rats following a challenge with a submaximal standard diuretic dose of the κ -agonist, U50,488. Thus, contrary to previous studies, suppression of neurohypophyseal vasopressin secretion

Figure 3 Effect of U50,488 (10 mg kg^{-1} s.c., $n = 4$ pairs) and vehicle administration (0.154 M NaCl , $n = 4$ pairs) on the initial 2 h urine output (a) and sodium excretion (b) and on plasma corticosterone concentration (c) in conscious, saline loaded ($4 \text{ ml } 100 \text{ g}^{-1}$, orally) fasted (stippled bars) and unfasted (solid bars) APW rats. Statistical comparisons between fasted and unfasted animals receiving either U50,488 or vehicle are indicated by * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

does not appear to be primarily involved in κ -agonist-induced diuresis in the rat and that additional mechanisms may contribute to the diuretic effect.

Although AVP appears to be involved in the diuretic effects of U50,488, this clearly does not involve suppression of plasma levels. In the Brattleboro DI rat, which congenitally lacks AVP, U50,488 administration was associated with an antidiuresis, a response which is normally associated with μ -receptor agonists such as morphine (Huidobro-Toro, 1980), rather than κ -agonists. There is no evidence to suggest that U50,488 acts at μ -receptors, however there are several reports which suggest that κ -

agonists may interact with renal AVP receptors. Gavend *et al.* (1978) showed that cyclazocine, which is structurally similar to the κ -agonist ethylketocyclazocine (EKC), attenuated the antidiuretic response to exogenous AVP in water-loaded rats and suggested that cyclazocine antagonized the renal effects of AVP. Slizgi & Ludens (1982) showed that although EKC reduced plasma AVP levels to a similar extent as water loading (10 mg kg^{-1}), urine formation was disproportionately greater in EKC-treated rats than in the hydrated rats. These authors also suggested additional mechanisms were contributing to the diuretic effect possibly by attenuating AVP action in the kidney. To support this concept they showed that EKC inhibited AVP-stimulated water flow across toad bladder, which is a model of the late distal tubule and collecting duct (Slizgi & Ludens, 1982). More recently these workers and others have shown the presence of renal κ -opioid binding sites which indicates that κ -opioids may have some direct action at the kidney as well as a central site of action (Quirion *et al.*, 1983; Slizgi & Ludens, 1985). Certainly AVP appears to be required to facilitate the diuretic actions of U50,488 but further investigations are required to define the precise relationship between AVP and the κ -agonists.

This study thus provides further evidence for a peripheral site of action of κ -agonist-induced diuresis in the rat. We have previously reported that following adrenal demedullation in the rat, κ -opioid agonists were no longer capable of inducing diuresis (Blackburn *et al.*, 1986), though the renal diuretic response to furosemide and clonidine and the antidiuretic response to morphine and buprenorphine were not impaired.

Adrenal demedullation did not appear to compromise adrenocortical function, since comparable plasma corticosterone levels were evident in vehicle-injected sham and demedullated rats in this and the previous study (Blackburn *et al.*, 1986). The consistent increase in plasma corticosterone in response to U50,488 observed here and in previous studies (Lahti & Collins, 1982; VonVoitlander *et al.*, 1983; Eisenberg, 1985; Iyengar *et al.*, 1986), which can be blocked by naloxone (Iyengar *et al.*, 1985), has been considered part of the stress response evoked by κ -opioids (Iyengar *et al.*, 1986). Undoubtedly the stress of fasting in this study would appear to have exaggerated the steroid response to U50,488, while clearly the response was attenuated in demedullated animals. The great reduction in adrenal and plasma catecholamine concentrations (92–99%) in demedullated animals (Blackburn *et al.*, 1986) might be expected to influence the renal responses; however, it appears that κ -agonists do not induce diuresis via action on medullary catecholamine release, since

pretreatment with the β -adrenoceptor antagonist propranolol and the α_1 -adrenoceptor antagonist prazosin had no effect on tifludom-induced diuresis (Blackburn *et al.*, 1986). Although Birch & Hayes (1989) reported that the α_2 -adrenoceptor antagonist idazoxan (1 mg kg^{-1}) attenuated a κ -opioid induced diuresis, we did not observe such an effect at doses of 0.5 mg kg^{-1} (Blackburn *et al.*, 1986) or 2 mg kg^{-1} (unpublished observations). Whilst the adrenal medulla has been shown to contain AVP (Ang & Jenkins, 1984), it seems unlikely that this plays a major role in the κ -agonist-induced diuresis either, since demedullated rats maintained normal water and salt balance (Blackburn *et al.*, 1986). Indeed, plasma AVP levels in sham and demedullated groups were not different. Thus, the mechanisms involved in the attenuation of a κ -agonist induced diuresis by adrenal demedullation are not readily apparent, but the absence of the normally associated increase in plasma corticosterone might be important.

An important contribution of this study is the detailed description of the time course of U50,488's renal effects, which has revealed an action on both urinary water and electrolyte excretion. Previous studies have examined urine output data over a single 5 h collection period (e.g. Slizgi & Ludens, 1982; VonVoigtlander *et al.*, 1983; Huidobro-Toro & Parada, 1985) or expressed urine output as a cumulative value over 2 and 5 h (e.g. Leander, 1983a; Leander *et al.*, 1985). The data described in the present study have shown that the diuretic activity of s.c. administered U50,488 is limited to the first 3 h post-injection, and that this is associated with an antinatriuresis and antikaliuresis. We have previously demonstrated that both the diuretic and antinatriuretic actions of U50,488 are blocked by a high, but not a low, dose of naloxone in conscious (Blackburn *et al.*, 1986) and anaesthetized preparations (Balment *et al.*, 1986), which suggest that these effects are mediated through the κ -receptor. Slizgi *et al.* (1984) found that U50,488 caused a slight decrease in sodium excretion and had no effect on potassium in the anaesthetized dog, but the dose employed was half that used in this study (5 mg kg^{-1} cf. 10 mg kg^{-1}). Huidobro-Toro & Parada (1985) noted a similar reduction in electrolyte excretion using the same dose of U50,488 (10 mg kg^{-1}) and proposed a role for endogenous κ -opioids in the regulation of both fluid and electrolytes. The observation that U50,488 administration was associated

with raised renin activity, that dynorphin A-(1-10) amide stimulates secretion of atrial natriuretic peptide (ANP) (Xie *et al.*, 1988) and that other κ -agonists have been shown to stimulate corticosterone release (VonVoigtlander *et al.*, 1983, Fuller & Leander, 1984; Eisenberg, 1985; Iyengar *et al.*, 1986) suggests that changes in renal haemodynamics or glomerular filtration rate contribute to these observed changes in urinary sodium and water loss. The renal retention of sodium did not appear to be associated with major changes in plasma aldosterone levels.

Slizgi *et al.* (1984) suggested that the U50,488-induced diuresis may reflect a decrease in tubular reabsorption of water, but the associated increase in tubular fluid flow rate might be expected to result in a natriuresis rather than the observed retention of sodium, indicating perhaps that the effects of U50,488 on sodium excretion are mediated by another mechanism.

Whilst adrenal demedullation attenuated the diuretic effects of U50,488, it did not alter the antinatriuretic response. Sodium excretion rates were higher in demedullated groups, but the percentage fall in the excretion rate between vehicle and U50,488 groups was similar in sham (32%) and demedullated (30%) animals, suggesting that the factor(s) affected by demedullation may only influence the renal diuretic response to U50,488. Further support for the concept that the diuretic and antinatriuretic actions of U50,488 may be mediated separately comes from the observation that DI rats responded in a similar manner to AVP-replete APW rats by showing a fall in sodium excretion in response to U50,488, whilst undergoing an anti-diuresis rather than a diuresis.

In summary, we have shown by using a protocol of hourly urine sampling that the κ -agonist U50,488 exerts a diuretic effect in the hydrated rat for 3–4 h post-injection. This diuresis did not appear to be mediated by inhibition of AVP secretion, but could be attenuated by prior adrenal demedullation. In addition U50,488 administration was associated with an antinatriuresis and antikaliuresis, which appeared to be mediated via different mechanisms from those involved in the diuretic response.

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