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# Mechanism of diuretic action of spiradoline (U-62066E)—a kappa opioid receptor agonist in the human

G. H. RIMOY, N. K. BHASKAR, D. M. WRIGHT[1] & P. C. RUBIN
Department of Therapeutics, Queen's Medical Centre, Nottingham NG7 2UH and [1]Upjohn Limited, Fleming Way, Crawley, West Sussex RH10 2NJ

1 The mechanism of the diuretic effect of the kappa opioid receptor agonist spiradoline was investigated in 10 healthy male subjects in a placebo-controlled, double-blind cross-over study.
2 Urine volume and osmolality, plasma vasopressin and Doppler renal blood velocity indices were recorded for 1.25 h before and 6 h following injection.
3 Spiradoline caused a significant increase in urine output which was antagonized by high but not low dose naloxone. The urine increase was accompanied by a significant decrease in osmolality which was also antagonised by high but not low dose naloxone.
4 Spiradoline had no effect on plasma vasopressin concentration or on renal blood velocity indices.
5 We conclude that kappa agonists induce diuresis in humans by a mechanism not involving suppression of vasopressin or changes in renal blood velocity indices.

**Keywords** spiradoline U-62066E naloxone diuresis plasma vasopressin Doppler renal blood flow

## Introduction

Opioid drugs interact with specific receptors which are classified into mu, delta and kappa (Martin *et al.*, 1976). Mu agonists such as morphine and methadone cause antidiuresis in hydrated animals and humans (De Bodo, 1944). Delta-opioid agonists such as metkephamid cause some diuresis in the human (Zerbe *et al.*, 1982) but kappa agonists such as ethyketocyclazocine and U-50488H substantially increase urine output and decrease urine osmolality without changing electrolyte excretion in several species including humans (Slizgi & Ludens, 1982). Brattleboro rats which lack endogenous ADH show no diuresis with kappa agonists (Leander, 1983b) and administration of the vasopressin analogue desmopressin completely abolished the diuretic effect of kappa agonists in the rat (Leander *et al.*, 1985). It has therefore been hypothesized that suppression of vasopressin is the main mechanism to account for the diuretic actions of kappa opioid agonists. Several studies in experimental animals (Leander, 1985; Slizgi & Ludens, 1982; Yamada *et al.*, 1989) have shown that kappa agonists decrease plasma vasopressin levels while others (Ashton *et al.*, 1989; Lahti *et al.*, 1985) showed no effect.

Spiradoline, U-62066E, ([5α, 7α, 8β]-(±)-3,4 dichloro-*N*-(7-(pyrolidinyl)-1-oxaspiro (4,5) dec-8-yl] benzeneacetamide methane sulphonate salt) is a highly selective kappa opioid receptor agonist structurally related to U-50488H (Lahti *et al.*, 1985).

The purpose of the present study was to study further the mechanism of the diuretic action of spiradoline in man.

## Methods

### *Subjects*

Ten healthy male volunteers, aged 21–29 years participated in the study which was approved by the University Medical School Ethics Committee and for which each volunteer gave written informed consent. All volunteers were non-smokers and were instructed not to take coffee or alcohol 24 h prior to each study day.

### *Study design*

The study was based on a randomised, double-blind four part placebo-controlled cross-over design. The volunteers attended the Department of Therapeutics at 07.00 h on study day 1 after an overnight fast. They were

Correspondence: Professor P. C. Rubin, Department of Therapeutics, Queen's Medical Centre, Nottingham NG7 2UH

given tap water (5 ml $kg^{-1}$ body weight) to drink and immediately emptied the bladder, an aliquot being retained for osmolality measurement.

Subsequently an intravenous cannula was inserted in the non-dominant arm. Baseline measurements were made during the 1.25 h prior to administration of the study drug. Subjects received in random order the following treatments with 1 week separating each cross-over:

1) placebo i.v. + placebo i.m.
2) placebo i.v. + spiradoline i.m.
3) naloxone 1 i.v. + spiradoline i.m.
4) naloxone 2 i.v. + spiradoline i.m.

Placebo i.v. and placebo i.m. were respectively naloxone and spiradoline vehicle; spiradoline was administered as a single dose of 3.2 $\mu g\ kg^{-1}$ free base (4.0 $\mu g\ kg^{-1}$ salt); naloxone was administered at doses of 0.012 mg $kg^{-1}$ (naloxone 1) and 0.2 mg $kg^{-1}$ (naloxone 2) in order to differentiate mu and kappa opioid effects. Volume blindness was maintained by all intramuscular injections being given at a constant dose volume for individual subjects, this being the volume required for the highest naloxone dose. Light meals and drinks were allowed 3 h after administration of the drug.

*Plasma assays*

Blood samples for the assay of plasma vasopressin were drawn from the indwelling cannula at 1.5 h and 0.75 h before drug injection and at 0.3, 1.2, 1.6 and 2.4 h post injection. Heparin was injected into the cannula after every withdrawal of blood and was removed before blood withdrawal. Blood was drawn into chilled syringes and transferred immediately into chilled lithium-heparin tubes and placed into ice and protected from light with cellophane paper. The samples were centrifuged within 5 min at 4° C and 3500 rev $min^{-1}$ for 8 min and the plasma separated and stored at −40° C until analysis. Plasma vasopressin was measured by radioimmunoassay in the Medical Research Council Blood Pressure Unit, Glasgow. The within and between assay coefficient of variation was 6.1% and 10.8% respectively. The lower limit of detection was 0.05 pg $ml^{-1}$ [normal range: 0.2–0.7 pg $ml^{-1}$ with water load (20 ml $kg^{-1}$) or 0.6–3.1 pg $ml^{-1}$ with 24 h water restriction].

*Urine collection*

Urine collection was repeated immediately before drug injection and 2 h after dosing. The volumes were measured and a sample retained for osmolality measurement.

*Doppler studies*

Renal blood flow was measured using Duplex Doppler (Doptek, Chichester, West Sussex, UK) with a 2.0 MHz pulsed transducer. The right renal artery was insonated from the right posterior flank with the subject lying on his left side and the probe directed cranially. The location of the probe was 6–8 cm from the spinous processes and 3–4 cm below the last rib and the measurement depth varied from 8.6–10 cm. When the characteristic signal—one with a rapid upstroke in systole and high diastolic flow likened to a 'ski' slope (Dubbins, 1986)—was obtained, the subject was instructed to briefly hold his breath and the signal recorded on to tape.

Occasionally because of respiratory movements several attempts were undertaken before a good signal could be recorded. Pulsatility index (PI) and peak flow velocity were analysed from the Doppler waveforms.

PI is defined as

$$PI = \frac{A-B}{mean}$$

where A is the maximum, B is the minimum and mean is the time-average of the maximum Doppler shift frequency over the cardiac cycle (Gosling & King, 1974). PI is widely accepted as an index of downstream impedance—the higher the PI the higher the resistance and vice versa (Burns, 1987). Peak flow velocity represents the maximum frequency shift achieved during systole. The between day coefficient of variation of the Doppler technique has previously been studied in our laboratory and found not to exceed 15% (Rimoy *et al.*, 1991).

Renal blood flow measurements were undertaken at 1.25 and 0.5 h prior to drug injection and at 0.5, 1.25, 2, 2.75, 4, 5 and 6 h after dosing.

*Statistical analysis*

All data are expressed as mean ± s.d. Two-way analysis of variance with repeated measures was used to assess changes from baseline at each follow-up interval to compare differences between placebo and the other three treatment groups. Paired *t*-test was used to compare the treatment with placebo on urine volume. Friedman two-way analysis of variance was used to assess the urine osmolality before and after the various treatments.

## Results

Urine osmolality decreased significantly with hydration in all the treatment groups (Figure 1). The osmolality following injection did not change following placebo but decreased significantly following spiradoline and spira-

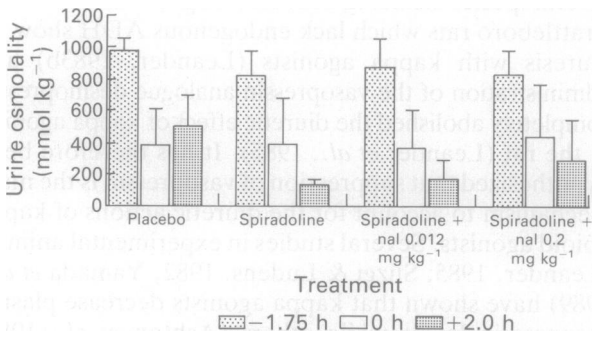


**Figure 1** Urine osmolality (mean ± s.d.) immediately preceding injection was similar in all four groups. Following placebo, urine osmolality increased while following spiradoline alone it fell still further ($P < 0.0002$ *c.f.* placebo). Urine osmolality following spiradoline + high dose naloxone was greater compared with spiradoline alone ($P < 0.06$). Low dose naloxone did not attenuate the action of spiradoline.

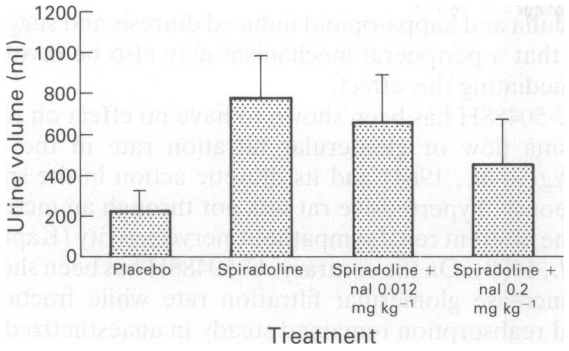


**Figure 2** Urine output (mean ± s.d.) increased significantly with spiradoline ($P < 0.0001$). The urine output following spiradoline + low dose naloxone was the same as high with spiradoline alone ($P > 0.1$). However, spiradoline + high dose naloxone produced a urine output significantly less than with spiradoline alone ($P < 0.0004$).

doline with low dose naloxone. High dose naloxone attenuated but did not abolish the decrease in osmolality caused by spiradoline.

Spiradoline increased urine output significantly (Figure 2). This effect was not influenced by low dose naloxone but was attenuated by high dose naloxone.

Spiradoline did not have any statistically significant effect on plasma vasopressin concentrations (Table 1).

Spiradoline had no significant effect on either pulsatility index or peak flow velocity in the renal artery (Table 2).

## Discussion

This study has confirmed the ability of selective kappa agonists to induce diuresis but in man has not confirmed

**Table 1** Influence of spiradoline on plasma arginine vasopressin (AVP) concentrations (pg $ml^{-1}$) (mean ± s.d., $n = 10$)

| *Time (h)* | *Placebo* | *Spiradoline* | *Spiradoline + naloxone$_1$* | *Spiradoline + naloxone$_2$* |
|---|---|---|---|---|
| −1.5 | 0.20 ± 0.22 | 0.19 ± 0.18 | 0.17 ± 0.09 | 0.14 ± 0.06 |
| −0.75 | 0.13 ± 0.08 | 0.15 ± 0.07 | 0.17 ± 0.12 | 0.25 ± 0.21 |
| +0.3 | 0.27 ± 0.35 | 0.20 ± 0.32 | 0.19 ± 0.22 | 0.20 ± 0.23 |
| +1.2 | 0.20 ± 0.26 | 0.15 ± 0.11 | 0.31 ± 0.57 | 0.21 ± 0.18 |
| +1.6 | 0.30 ± 0.37 | 0.42 ± 0.40 | 0.49 ± 0.37 | 0.25 ± 0.18 |
| +2.4 | 0.22 ± 0.34 | 0.33 ± 0.38 | 0.23 ± 0.20 | 0.29 ± 0.41 |

Naloxone$_1$—0.012 mg $kg^{-1}$
Naloxone$_2$—0.2 mg $kg^{-1}$.

**Table 2** Influence of spiradoline on renal blood flow (mean ± s.d., $n = 10$)

| | *Time (h)* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *−1.25* | *−0.5* | *+0.5* | *+1.25* | *+2* | *+2.75* | *+4* | *+5* | *+6* |
| *Pulsatility index* | | | | | | | | | |
| Placebo | 1.08 | 1.12 | 1.14 | 1.13 | 1.13 | 1.14 | 1.20 | 1.16 | 1.16 |
| (± s.d.) | 0.16 | 0.13 | 0.13 | 0.14 | 0.13 | 0.15 | 0.09 | 0.14 | 0.13 |
| Spiradoline | 1.16 | 1.16 | 1.14 | 1.16 | 1.15 | 1.12 | 1.21 | 1.16 | 1.14 |
| (± s.d.) | 0.16 | 0.13 | 0.12 | 0.13 | 0.14 | 0.11 | 0.16 | 0.13 | 0.13 |
| Spiradoline + naloxone 0.012 mg $kg^{-1}$ | 1.13 | 1.07 | 1.09 | 1.13 | 1.13 | 1.10 | 1.19 | 1.20 | 1.15 |
| (± s.d.) | 0.11 | 0.16 | 0.14 | 0.13 | 0.13 | 0.15 | 0.11 | 0.14 | 0.14 |
| Spiradoline + naloxone 0.2 mg $kg^{-1}$ | 1.19 | 1.13 | 1.14 | 1.20 | 1.16 | 1.15 | 1.23 | 1.09 | 1.20 |
| (± s.d.) | 0.12 | 0.14 | 0.13 | 0.11 | 0.13 | 0.10 | 0.11 | 0.10 | 0.12 |
| *Peak flow velocity ($cms^{-1}$)* | | | | | | | | | |
| Placebo | 65.10 | 65.60 | 69.80 | 69.10 | 69.40 | 69.10 | 69.40 | 69.10 | 69.00 |
| (± s.d.) | 8.41 | 9.26 | 11.72 | 12.26 | 10.82 | 10.15 | 14.65 | 10.13 | 12.71 |
| Spiradoline | 68.20 | 67.50 | 67.30 | 68.00 | 70.70 | 69.40 | 75.20 | 70.20 | 72.80 |
| (± s.d.) | 12.07 | 10.2,5 | 12.12 | 13.48 | 11.04 | 10.09 | 13.37 | 14.27 | 12.64 |
| Spiradoline + naloxone 0.012 mg $kg^{-1}$ | 74.96 | 76.40 | 71.80 | 70.70 | 72.30 | 74.50 | 71.80 | 77.40 | 77.80 |
| (± s.d.) | 23.11 | 20.14 | 18.31 | 16.38 | 14.34 | 18.95 | 18.10 | 20.01 | 18.41 |
| Spiradoline + naloxone 0.2 mg $kg^{-1}$ | 69.30 | 64.60 | 64.60 | 65.70 | 70.30 | 66.40 | 67.80 | 67.20 | 66.80 |
| (± s.d.) | 12.52 | 13.24 | 11.61 | 12.44 | 10.97 | 10.94 | 8.30 | 9.77 | 10.10 |

the hypothesis that the mechanism involves suppression of vasopressin. Spiradoline increased the urine output and decreased the osmolality as previously reported in the human (Peters *et al.*, 1987). These effects were antagonized by high but not low dose naloxone suggesting an action via an opioid receptor but not of the mu-subtype.

Inhibition of vasopressin release has been reported to be the major mechanism for the diuretic action of spiradoline in rats (Yamada *et al.*, 1989, 1990) but our results do not support this as the major mechanism in man. There is other evidence to suggest that suppression of vasopressin does not entirely account for the diuretic action of kappa agonists. In the rat bremazocine at doses of 0.02 mg $kg^{-1}$ suppresses plasma vasopressin concentrations but at a dose of 0.005 mg $kg^{-1}$ does not suppress vasopressin while still causing a diuresis (Leander, 1983a,b; Leander *et al.*, 1985). In addition, kappa agonists antagonize the action of exogenous vasopressin administered to water-loaded rats (Gavend *et al.*, 1978) and Brattleboro rats (Slizgi & Ludens, 1986). It has also been reported that ethylketocyclazocine blocks the vasopressin-stimulated water flow in the toad urinary bladder (Slizgi & Ludens, 1982). Based on the demonstration of kappa opioid binding sites in the kidney (Quirion *et al.*, 1983; Slizgi & Ludens, 1985), it has been suggested that kappa agonists have a direct renal action. At the same time it has been shown in rats that bilateral adrenal demedullation significantly attenuated the diuretic responses to the kappa-opioid agonists U-50488H, ethylketocyclazocine and tifluadom, but did not affect basal urine output, frusemide-induced diuresis or the antidiuretic response to morphine and buprenorphine (Ashton *et al.*, 1989; Blackburn *et al.*, 1986; Borkowski, 1989) indicating a link between the adrenal medulla and kappa-opioid induced diuresis and suggesting that a peripheral mechanism may also be involved in mediating this effect.

U-50488H has been shown to have no effect on renal plasma flow or glomerular filtration rate in the dog (Slizgi *et al.*, 1984) and its diuretic action in the spontaneously hypertensive rat was not through an increase in the efferent renal sympathetic nerve activity (Kapusta *et al.*, 1989). On the contrary, U-50488H has been shown to increase glomerular filtration rate while fractional fluid reabsorption remained steady in anaesthetized rat (Ashton *et al.*, 1990) and increase plasma corticosterone levels in conscious rat (Ashton *et al.*, 1989), hence the increase in glomerular filtration rate may be mediated through an increase in angiotensin II levels and the action of the plasma corticosterone. In our study spiradoline did not have any significant effect on either pulsatility index or peak flow velocity in the renal artery implying that renal haemodynamic changes are unlikely to account for the diuresis observed.

In conclusion, this study has shown that spiradoline causes a marked diuresis in man which, being reversible by naloxone only at high doses, is likely to be an opioid kappa receptor effect. Suppression of vasopressin release or changes in renal haemodynamics are unlikely to be the mechanism underlying these effects since no changes in circulating levels of vasopressin or renal blood velocity indices were observed. It is possible that spiradoline modulates the peripheral action of vasopressin in man.

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## References

Ashton, N., Balment, R. J. & Blackburn, T. P. (1989). K-opioid induced changes in renal water and electrolyte management and endocrine secretion. *Br. J. Pharmac.*, **97**, 769–776.

Ashton, N., Balment, R. J. & Blackburn, T. P. (1990). K-opioid receptor agonists modulate the renal excretion of water and electrolytes in anaesthetized rats. *Br. J. Pharmac.*, **99**, 181–185.

Blackburn, T. P., Borkowski, K. R., Friend, J. & Rance, M. J. (1986). On the mechanism of K-opioid diuresis. *Br. J. Pharmac.*, **89**, 593–598.

Borkowski, K. R. (1989). Studies on the adrenomedullary dependence of K-opioid agonist-induced diuresis in conscious rats. *Br. J. Pharmac.*, **98**, 1151–1156.

Burns, P. N. (1987). The physical principles of Doppler and spectral analysis. *J. clin. Ultrasound*, **15**, 567–590.

De Bodo, R. C. (1944). The antidiuretic action of morphine and its mechanism. *J. Pharmac. exp. Ther.*, **82**, 74–88.

Dubbins, P. A. (1986). Renal artery stenosis: Duplex Doppler evaluation. *Br. J. Radiology*, **59**, 225–229.

Gavend, M., Gavend, M. R. & Serre, F. (1978). Etude experimentale de l'action diuretique d'un antagoniste des morphiniques. *J. Pharmac.* (Paris), **9**, 77.

Gosling, R. G. & King, D. H. (1974). Continuous wave ultrasound as an alternative and complement to X-rays in vascular examinations. In *Cardiovascular applications of ultrasound*, ed. Reneman, R. S. pp. 266–282. North-Holland, Amsterdam: Elsevier.

Kapusta, D. R., Jones, S. Y. & DiBona, G. F. (1989). Role of renal nerves in excretory responses to administration of kappa agonists in conscious spontaneously hypertensive rats. *J. Pharmac. exp. Ther.*, **251**, 230–237.

Lahti, R. A., Michelson, M. M., McCall, J. M. & Von Voigtlander, P. F. (1985). [$^3$H] U-69593: a highly selective ligand for the opioid K-receptor. *Eur. J. Pharmac.*, **109**, 281–284.

Leander, J. D. (1983a). A kappa opioid effect: Inoreased urination in the rat. *J. Pharmac. exp. Ther.*, **224**, 89–94.

Leander, J. D. (1983b). Further study of kappa opioids on increased urination. *J. Pharmac. exp. Ther.*, **227**, 35–41.

Leander, J. D., Hart, J. C. & Zerbe, R. L. (1985). Diuresis and suppression of vasopressin by kappa-opioids: comparison with mu- and delta-opioids and clonidine. *J. Pharmac. exp. Ther.*, **234**, 463–469.

Martin, W. R., Eades, G. G., Thompson, J. A., Huppler, R. E. & Gilbert, P. E. (1976). The effects of morphine- and nalorphine-like drugs in non-dependent and morphine-dependent chronic spinal dogs. *J. Pharmac. exp. Ther.*, **197**, 517–532.

Peters, G. R., Ward, N. J., Antal, E. G., Lai, D. Y. &

DeMaar, E. W. (1987). Diuretic actions in man of a selective kappa opioid agonist: U-62066E. *J. Pharmac. exp. Ther.*, **240**, 128–131.

Quirion, R., Finkel, M. S., Mendelsohn, F. A. O. & Zamir, N. (1983). Localization of opiate binding sites in kidney and adrenal gland of the rat. *Life Sci.*, **33**, 299–302.

Rimoy, G. H., Bhaskar, N. K. & Rubin, P. C. (1991). Reproducibility of Doppler blood flow velocity waveform measurements. *Eur. J clin. Pharmac.*, (in press).

Slizgi, G. R. & Ludens, J. H. (1982). Studies on the nature and mechanism of the diuretic activity of the opioid analgesic ethyketocyclazocine. *J. Pharmac. exp. Ther.*, **220**, 585–591.

Slizgi, G. R. & Ludens, J. H. (1985). Displacement of 3H-EKC binding by opioids in rat kidney: A correlate to diuretic activity. *Life Sci.*, **36**, 2189–2193.

Slizgi, G. R. & Ludens, J. H. (1986). Role of ADH in ethylketocyclazocine-induced diuresis: studies in the Brattleboro rat. *Life Sci.*, **38**, 2437–2440.

Slizgi, G. R., Taylor, C. J. & Ludens, J. H. (1984). Effects of the highly selective kappa opioid U-50488, on renal function in the anaesthetized dog. *J. Pharmac. exp. Ther.*, **230**, 641–645.

Wilson, N. & Ngsee, J. (1982). Antidiuretic effect of acute morphine administration in the conscious rat. *Can. J. Physiol. Pharmac.*, **60**, 201–204.

Yamada, K., Imai, M. & Yoshida, S. (1989). Mechanism of diuretic action of U-62066E, a K-opioid receptor agonist. *Eur. J. Pharmac.*, **160**, 229–237.

Yamada, K., Nakamo, M. & Yoshida, S. (1990). Inhibition of elevated arginine vasopressin secretion in response to osmotic stimulation and acute haemorrhage by U-62066E a kappa-opioid receptor agonist. *Br. J. Pharmac.*, **99**, 384–388.

Zerbe, R., Henry, D. P. & Robertson, G. L. (1982). A new metenkephalin analogue suppresses plasma vasopressin in man. *Peptides*, **1**, 199–201.

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