**British Journal of Pharmacology (1997) 120,** 781 – 784 

# The effects of spiradoline (U-62066E), a $\kappa$-opioid receptor agonist, on neuroendocrine function in man

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**1** Opioid drugs act on specific receptors which are principally classified into $\mu$, $\delta$ and $\kappa$ subtypes. Spiradoline (U-62066E) is a $\kappa$-selective agent which has been shown to possess potent anti-nociceptive effects but does not show cross tolerance with morphine.

**2** We have assessed the neuroendocrine effects of spiradoline in healthy volunteers with two doses (1.6 and 4.0 $\mu$g kg$^{-1}$, i.m.) of the compound. Six male non-smokers aged 19 – 27 years were studied by use of a randomized, double-blind three-limb placebo-controlled cross-over design. Blood was taken from an in-dwelling venous cannula basally and at 15 min intervals for 2 h for determination of serum cortisol, prolactin, growth hormone (GH) and catecholamines.

**3** Psychological function was assessed by the Stanford Sleepiness Scale (SSS) and the Addiction Research Centre Inventory (ARCI) administered before the medication and at 35 min, 1 h 25 min and 2 h afterwards. Cardiovascular variables were recorded at 10 min intervals. Results were analysed by analysis of variance.

**4** Spiradoline showed a significant ($P<0.05$) dose-dependent increase in free water clearance, as predicted for a $\kappa$-opioid agonist. It also caused a dose-dependent stimulation of prolactin, (increment over baseline for higher dose 214%), GH (433%) and cortisol (215%) release ($P<0.05$). There were no significant drug-related changes in plasma catecholamines, blood pressure, pulse or psychological variables.

**5** We have therefore confirmed that $\kappa$-opioids increase free-water clearance and may participate in the stimulation of prolactin and GH release. In contrast to $\mu$ and $\delta$-opioid agonists, this novel $\kappa$-agonist stimulates cortisol release in man.

**Keywords:** Spiradoline; $\kappa$-opioid agonist; opioid receptors; hypothalamo-pituitary-adrenal axis; prolactin; growth hormone

## Introduction

Endogenous opioid peptides are known to play a major role in the regulation of pituitary hormone secretion (van Wimersma Greidanus & Grossman, 1991). Pharmacological studies have provided evidence for the presence of multiple opioid receptor subtypes, of which the best characterized are the $\mu$, $\delta$ and $\kappa$ categories. The role of $\mu$ and $\delta$ receptor subtypes in the mediation of opiate effects on neuroendocrine function has been extensively studied. Stimulation of $\mu$ receptors is associated with increased secretion of prolactin and thyroid stimulating hormone (TSH); some $\mu$-agonists, although paradoxically not morphine itself, stimulate growth hormone (GH) release. There is also substantial evidence in favour of $\mu$-receptor-mediated inhibition of gonadotrophin release (Rotsztejn *et al*., 1978). However, autoradiographic studies have shown that the predominant opioid receptors in the hypothalamic supraoptic and paraventricular nuclei, and in the neurohypophysis, are mainly of the $\kappa$ subtype (Simantov & Snyder, 1977; Mansour *et al*., 1988). In recent years, the introduction of a number of selective $\kappa$-agonists has allowed for the characterization of such receptor activity in laboratory animals (Hamon & Jouquey, 1990). In addition to analgesic effects (Kunihara *et al*., 1992), $\kappa$-opioids have been shown to block milk ejection (Wright & Clark, 1986) and established parturition (Douglas *et al*., 1993) in the rat through inhibition of oxytocin release. They have also been shown to decrease plasma vasopressin levels (Leander *et al*., 1985). However, determining the role of $\kappa$-opioids in man has been hampered by the absence, until recently, of selective $\kappa$-agonists available for clinical use. Spiradoline mesylate (U-62066E, ([5$\alpha$,7$\alpha$,8$\beta$]-($\pm$)-3,4 dichloro-N-(7-pyrolidinyl)-1-oxaspiro(4,5)dec-8-yl)) is a $\kappa$-selective agent which has been shown to possess potent anti-nociceptive effects but does not show any cross-tolerance with morphine. Spiradoline is 84 times more selective for the $\kappa$-receptor than the $\mu$- or $\delta$-receptors (Lahti *et al*., 1985) and has been used as a highly selective $\kappa$ agonist for human studies (Wright *et al*., 1991). In the present study we have, therefore, set out to assess the neuroendocrine effects of spiradoline in healthy human volunteers. A preliminary account of our findings has been published (Ur *et al*., 1993).

## Methods

Six healthy male subjects aged 19 – 27 years were studied by use of a randomized, double-blind three-limb placebo-controlled cross-over design. All were non-smokers and were asked to refrain from alcohol and 'over the counter' medications within 24 h of each study day. Following approval by the St. Bartholomew's Hospital Ethics Committee, subjects gave full informed written consent and were studied at weekly intervals in our clinical investigation unit. For each session, after overnight fast the subject was admitted at 08.30 h, placed sitting into an upright chair, and then an in-dwelling venous cannula was inserted into a non-dominant forearm vein one hour before the start of the study. They were required to drink 5 ml kg$^{-1}$ tap water, and then drug (either 1.6 or 4.0 $\mu$g kg$^{-1}$ spiradoline mesylate; obtained from Upjohn, Kalamazoo, MI, U.S.A.) or placebo (0.9% saline) was administered as a 1 ml intramuscular injection, following which blood was taken basally and at 15 min intervals for 105 min for determination of serum cortisol, prolactin, GH and catecholamine concentrations. Urine was collected before the injection of the drug and

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then after two hours for determination of total volume passed and osmolality. Cardiovascular variables (BP and pulse) were recorded at 10 min intervals throughout the study. Psychological function was assessed by well-validated standardized self-administered questionnaires: the Stanford Sleepiness Scale (SSS) and the Addiction Research Centre Inventory (ARCI) which were given to the subjects before the medication and at 35 min, 1 h 25 min and 2 h afterwards.

*Assays*

Serum was separated after clotting and assayed for cortisol, prolactin and GH. Serum cortisol was measured by radioimmunoassay (RIA) (Cunnah *et al*., 1987) with an intra-assay coefficient of variation (CV) of 6% Serum prolactin was measured by RIA (Edwards, 1983) and standardized against MRC 75/504 with a normal range $<360$ m$\mu$ l$^{-1}$. The intra-assay CV was 8.5%. Serum levels of GH were measured by an established in-house non-extracted RIA validated against the North East Thames Quality Assurance Scheme, and standardized against preparation 66/217 obtained from the National Institute of Biological Standards and Control (NIBSC, South Mimms, Herts.). The intra-assay CV for this assay was 5.5%. Plasma for catecholamines was separated at 4°C, extracted on alumina, and measured by a well-established high performance liquid chromatographic (h.p.l.c.)/electrochemical detection technique (Bouloux *et al*., 1985). The intra-assay CV was 1.5% for noradrenaline, 1.7% for adrenaline and 0.8% for dopamine. Urine and plasma osmolality were measured by depression of freezing point (Advanced Systems Osmometer, Advanced Instruments Inc., Needham Heights, Mass., U.S.A.).

*Statistical analysis*

The data were subjected to two-way analysis of variance with allowance for multiple non-independent samples. All results are given as means $\pm$ s.e.mean ($n=6$); unless otherwise stated significance was taken as $P<0.05$.

## Results

Spiradoline showed a significant dose-dependent increase in free water clearance (Figure 1). Spiradoline caused a dose-dependent stimulation of prolactin, increment of 214% with a peak at 60 min (Figure 2), cortisol, 433% at 45 min (Figure 3) and GH, 215% at 75 min (Figure 4). There were no significant drug-related changes in plasma catecholamines, blood pressure or pulse (data not shown).

With regard to the psychological parameters, there was no significant effect on sleepiness as assessed by SSS with increasing doses of spiradoline (Figure 5). On analysis of ARCI data there was a marked trend for a dose-dependent dysphoric effect, although this failed to achieve statistical significance (Figure 6); this was unrelated to the hormonal changes, and no effect on euphoria parameters was seen (Figure 7). Linear regression analysis revealed no significant correlation between the dysphoria and any of the neuroendocrine or cardiovascular parameters.

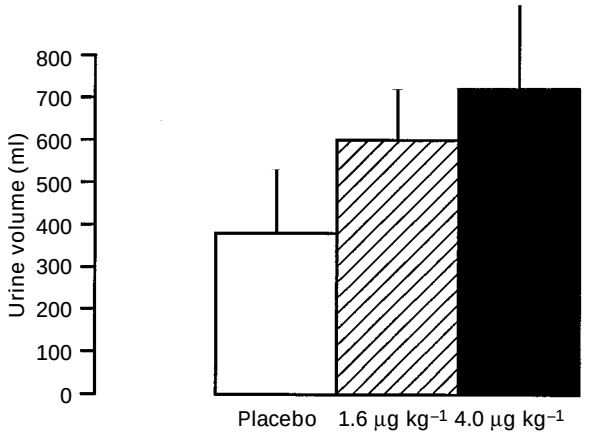


**Figure 1** Effect of spiradoline 1.6 $\mu$g kg$^{-1}$, i.m. (hatched column), 4.0 $\mu$g kg$^{-1}$, i.m. (solid column) and placebo (open column) on free water clearance in 6 healthy male subjects (mean $\pm$ s.e.mean).

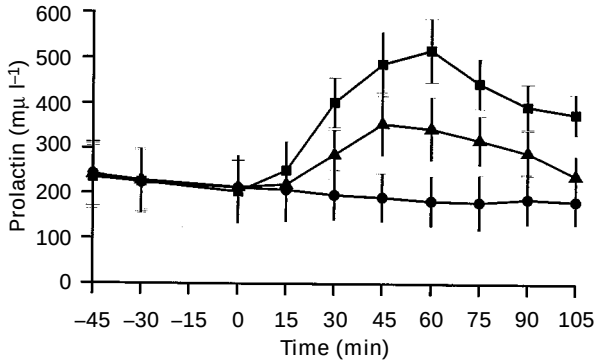


**Figure 2** Effect of spiradoline 1.6 $\mu$g kg$^{-1}$, i.m. (▲), 4.0 $\mu$g kg$^{-1}$, i.m. (■) and placebo (●) on serum prolactin levels in 6 healthy male subjects. Means are shown with vertical lines indicating s.e.mean.

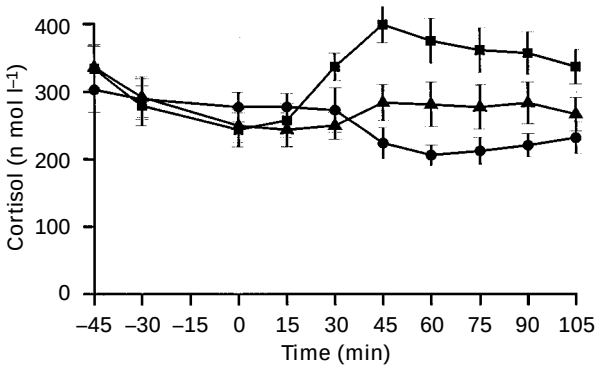


**Figure 3** Effect of spiradoline 1.6 $\mu$g kg$^{-1}$, i.m. (▲), 4.0 $\mu$g kg$^{-1}$, i.m. (■) and placebo (●) on serum cortisol levels in 6 healthy male subjects. Means are shown with vertical lines indicating s.e.mean.

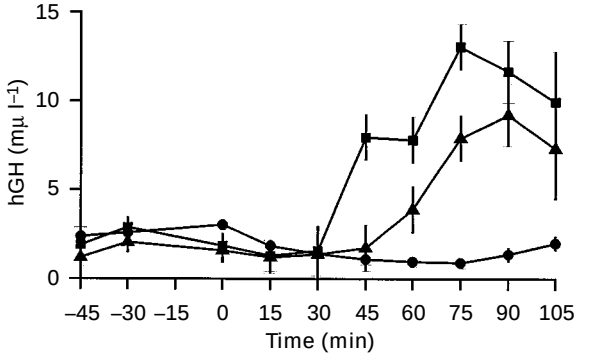


**Figure 4** Effect of spiradoline 1.6 $\mu$g kg$^{-1}$, i.m. (▲), 4.0 $\mu$g kg$^{-1}$, i.m. (■) and placebo (●) on serum growth hormone levels (hGH) in 6 healthy male subjects. Means are shown with vertical lines indicating s.e.mean.

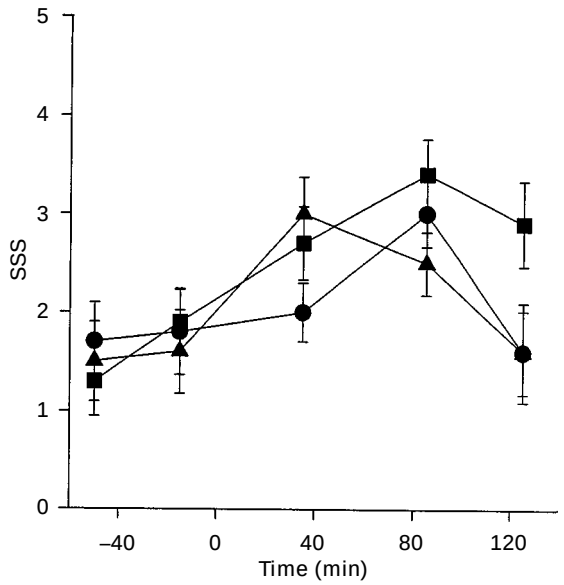


**Figure 5** Effect of spiradoline 1.6 $\mu$g kg$^{-1}$, i.m. (▲), 4.0 $\mu$g kg$^{-1}$, i.m. (■) and placebo (●) on Stanford Sleepiness Scale in 6 healthy male subjects. Means are shown with vertical lines indicating s.e.mean.

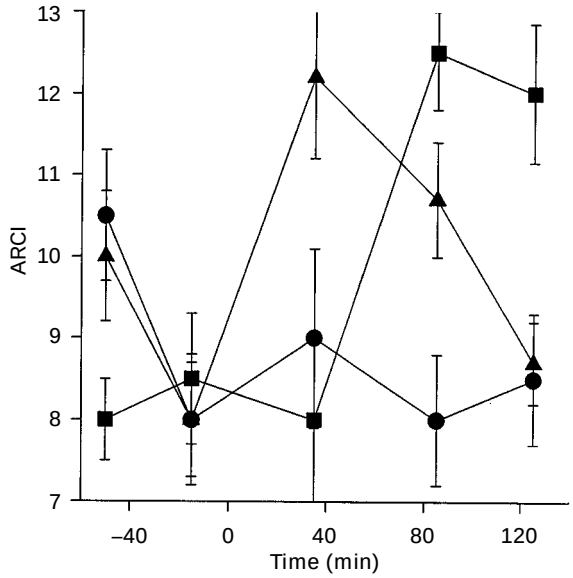


**Figure 7** Effect of spiradoline 1.6 $\mu$g kg$^{-1}$, i.m. (▲), 4.0 $\mu$g kg$^{-1}$, (■) and placebo (●) on euphoria measurement (ARCI) in 6 healthy male subjects. Means are shown with vertical lines indicating s.e.mean.

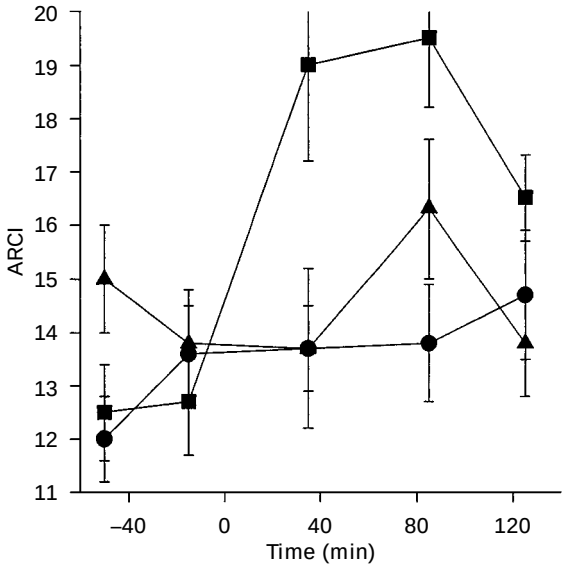


**Figure 6** Effect of spiradoline 1.6 $\mu$g kg$^{-1}$, i.m. (▲), 4.0 $\mu$g kg$^{-1}$, i.m. (■) and placebo (●) on dysphoria measurement (ARCI) in 6 healthy male subjects. Means are shown with vertical lines indicating s.e.mean.

## Discussion

We confirmed that the κ-opioid spiradoline increases free-water clearance in normal subjects, in keeping with previous results with this compound and related agents (Peters *et al*., 1987; Rimoy *et al*., 1991). In animals, it appears that the ability of these compounds to induce a diuresis is correlated with their degree of affinity for κ-receptors (Richards & Sadee, 1985; Slizgi & Ludens, 1985), and is inhibited by high doses of the non-selective opioid antagonist naloxone and low doses of the selective κ-antagonist, MR-2266 (Huidobro-Toro & Parada, 1985). In these studies the changes in plasma vasopressin were slight and not statistically significant, suggesting that such κ-mediated diuresis occurs through inhibition of the effect of vasopressin at the level of the renal tubule. However, in an earlier study of the enkephalin analogue, DAMME (Grossman *et al*., 1980), the opioid-induced diuresis was shown to be associated with a fall in circulating vasopressin levels only when these were artifically elevated by salt-loading. It therefore remains possible that the diuresis demonstrated is principally due to inhibition of vasopressin release, although a direct effect on the renal tubules cannot be excluded at the present time.

As with opioid peptides acting at μ-receptors, we have shown that spiradoline stimulates prolactin and GH release. Our data on prolactin are essentially in agreement with those of Chappell *et al*. (1993), who found a dose-dependent increase in prolactin in normal subjects and in individuals with Tourette's syndrome (a neurological condition characterized by repetitive stereotypic tics and vocalizations). However, we also noted a clear stimulation of GH release in our subjects, while in the previous study this was only seen in the Tourette patients. While the reasons for this discrepancy are not obvious, it renders it less likely that Tourette patients are selectively sensitive to certain of the neuroendocrine effects of spiradoline. Our data are also concordant with the results of Gilbeau *et al*. (1986) and Pfeiffer *et al*. (1986), who showed similar stimulation of prolactin and GH release in response to dynorphin in a non-human primate and to another κ-receptor agonist in man.

In contrast to μ and δ-opioid agonist, this novel κ-agonist appears to stimulate the adrenal system in man, with a dose-dependent increase in circulating cortisol. While we did not specifically measure adrenocorticotrophin (ACTH), most of the data in man suggest that opioid-induced changes in adrenocortical function are usually a manifestation of modulation, probably at the level of the hypothalamus. It is also likely that this effect is specific, as spiradoline caused no change in cardiovascular parameters. Mild dysphoric changes were noted in some subjects, but these did not correlate with the rise in cortisol. It has been previously suggested that endogenous opioids inhibit pituitary-adrenal activity by interacting with the noradrenergic input to corticotropin releasing hormone (CRH) and/or vasopressin (van Wimersma Greidanus & Grossman, 1991). These effects are relatively resistant to antagonism with naloxone, implicating the involvement of non-μ opiate receptors. Conversely, high doses of naloxone are required to demonstrate endogenous opioid inhibition of the hypothalamo-pituitary-adrenal axis. Recent data on the use of the highly selective δ-receptor agonist, deltorphin, suggest that the δ-receptors are the endogenous mediators of such re-

sponses (Degli Uberti *et al*., 1992). The present results suggest that κ-opiate receptors, unlike δ-receptors, are stimulators of the pituitary-adrenal axis in studies in man. These results are quite different from those obtained in studies on the rat, the majority of which support an inhibitory role for κ-opiate receptors to the pituitary-adrenal axis; thus, the release of CRH into the portal blood (Plotsky, 1986) or from hypothalamic explants (Tsagarakis *et al*., 1990) is potently suppressed by specific κ-receptor agonists. Thus, κ-receptors appear to increase HPA activity in man but inhibit it in the rat.

In summary, we have shown that spiradoline, a new highly selective κ-agonist drug, caused a dose-dependent stimulation of prolactin and GH release in human volunteers, while inducing an increase in free-water clearance. In contrast to μ- and δ-opioid receptor agonists, this κ-agonist appears to stimulate cortisol release in man. There were no significant drug-related changes in plasma catecholamines, blood pressure or pulse. There was a non-significant trend for a dose-dependent dysphoric effect which was unrelated to the hormonal changes.

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*(Received October 6, 1996*
*Accepted November 12, 1996)*