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Elevation of serum corticosterone in rats by bremazocine, a κ-opioid agonist

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Bremazocine at doses of 0·01 mg kg^{-1} s.c., and higher, increased serum corticosterone concentration several-fold in rats. The increase occurred within 20 min, was maximum at 40–60 min and subsided after 120 min. Pretreatment with naloxone (1–10 mg kg^{-1} s.c.) antagonized the corticosterone increase. These data support the view that κ-opioid agonist activity of bremazocine mediated the corticosterone increase.

Recently, Marko & Romer (1983) reported that bremazocine, a κ-opioid agonist, decreased serum luteinizing hormone (LH) levels in male and female rats, inhibited spontaneous ovulation in female rats, and decreased testosterone secretion in male rats. The decrease in LH induced by bremazocine, unlike that induced by morphine, was not antagonized by naloxone. We describe here another endocrine effect of bremazocine, elevation of serum corticosterone in rats, which occurs at low doses of bremazocine and is antagonized by naloxone.

Methods

Male Wistar rats from Harlan Industries, Cumberland, IN, were kept in at 24 °C room with 12 h light : dark cycles for at least one week and weighed 200–240 g at the time of the experiments. Bremazocine hydrochloride (Sandoz Ltd., Basle, Switzerland) and naloxone hydrochloride (Endo Laboratories, Garden City, NY) were dissolved in distilled water and injected subcutaneously; control rats received similar injections (1 ml kg^{-1}) of distilled water. Rats were decapitated, and trunk blood was collected and allowed to clot. Serum was collected after centrifugation and stored at −15 °C before analysis. Corticosterone was assayed spectrofluorometrically by the method of Solem & Brinck-Johnsen (1965). Results are expressed as mean values ± standard errors for 5 rats per group (except where 10 rats per group is specified), and comparisons between groups were made by Student's *t*-test.

Results

Table 1 shows that bremazocine increased serum corticosterone concentration rapidly after its s.c. injection into rats. The maximum increase was at 40–60 min. By 2 h, only a slight increase persisted, and by 4 h corticosterone concentration had returned to normal. Since the difference between bremazocine-treated and vehicle-treated groups was maximum at 60 min, the time used by Lahti & Collins (1982) in their studies of opioid agonist effects on corticosterone concentration, the 60-min treatment interval was used in subsequent experiments.

Table 2 shows the effect of five different doses of bremazocine on serum corticosterone concentration in rats. At 0·0001 mg kg^{-1} of bremazocine, there was no effect on serum corticosterone. At the 0·001 mg kg^{-1} dose, mean corticosterone concentration was increased but the effect was variable so that statistical significance was not reached. At 0·01 mg kg^{-1} and higher doses of bremazocine, a significant elevation of serum corticosterone was observed. The increase was 7-fold or greater compared to control values at each of the three highest doses.

Pretreatment with naloxone antagonized the elevation of serum corticosterone by bremazocine (Table 3).

Table 1. Time course for the elevation of serum corticosterone by bremazocine.

Time (min)	Serum corticosterone (μg/100 ml) Vehicle	Bremazocine
20	14·4 ± 2·9	27·1 ± 4·4*
40	14·8 ± 3·8	52·1 ± 2·3*
60	12·2 ± 2·9	51·8 ± 3·3*
120	7·7 ± 1·2	12·0 ± 1·0*
240	5·5 ± 0·5	5·5 ± 0·3

* Significant difference from vehicle-treated group ($P < 0·05$).

Bremazocine hydrochloride (0·01 mg kg^{-1}) or distilled water (1 ml kg^{-1}) was injected s.c. Serum corticosterone concentration in rats that received no injection was 4·8 ± 0·4 μg/100 ml.

Table 2. Dose-dependent elevation of serum corticosterone by bremazocine.

Dose of bremazocine (mg kg^{-1} s.c.)	Number of rats	Serum corticosterone μg/100 ml
0·0	10	6·9 ± 0·5
0·0001	5	7·8 ± 0·9
0·001	5	22·2 ± 7·4
0·01	10	51·0 ± 3·5*
0·1	5	47·5 ± 2·3*
1·0	5	49·8 ± 1·8*

* Significant difference from control ($P < 0·05$).

Bremazocine hydrochloride was injected 1 h before rats were killed.

* Correspondence.

Bremazocine at 0·01 mg kg^{-1} increased corticosterone more than 7-fold in this experiment as in the first one. Naloxone at doses of 1 mg kg^{-1} and higher antagonized the effect of bremazocine by more than 50%, the ED50 dose of naloxone being estimated at 0·8 mg kg^{-1}.

Table 3. Antagonism by naloxone of the bremazocine-induced elevation of serum corticosterone.

Dose of naloxone (mg kg^{-1} s.c.)	Serum corticosterone, μg/100 ml Vehicle-treated	Bremazocine-treated
0·0	5·8 ± 0·3	43·5 ± 2·6*
0·3	5·7 ± 0·5	33·3 ± 5·6*
1·0	6·3 ± 0·4	22·5 ± 4·0*†
3·0	5·5 ± 0·3	13·8 ± 3·2*†
10·0	6·8 ± 0·6	13·8 ± 3·7†

* Significant elevation of serum corticosterone compared to vehicle-treated group ($P < 0·05$).

† Significant difference from group treated with bremazocine alone ($P < 0·05$).

Bremazocine hydrochloride was injected s.c. at 0·01 mg kg^{-1} 1 h before rats were killed and 15 min after naloxone hydrochloride.

Discussion

Bremazocine is a potent and selective κ-agonist (Romer et al 1980), and its elevation of serum corticosterone in rats at doses as low as 0·01 mg kg^{-1} seems likely to result from κ-receptor activation. The antagonism by naloxone confirms that opioid receptors are involved in the effect. These findings with bremazocine are compatible with the results of Lahti & Collins (1982), who studied several other opioid drugs that elevate serum corticosterone in rats. The high potency of U-50,488 ((±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzenacetamide) in their studies strengthens the idea that κ-receptors are involved in the effect, since that compound is also a selective κ-agonist (VonVoigtlander et al 1983). The present results with bremazocine occurred over the same dose range (0·005–0·08 mg kg^{-1}) which produces a marked diuretic effect, presumably by inhibiting release of vasopressin (antidiuretic hormone) (Leander 1983).

Bremazocine can interact with μ-receptors, but here it acts as an antagonist, precipitating abstinence-induced jumping in morphine-dependent mice and antagonizing morphine analgesia (VonVoigtlander et al 1983). Since bremazocine mimics rather than antagonizes the action of morphine (George & Way 1955; Lahti & Collins 1982) in elevating serum corticosterone concentration, different receptors must be involved than in morphine dependency and analgesia.

The neuroendocrine effect of bremazocine reported by Marko & Romer (1983), lowering of serum LH, was not blocked by naloxone. Possibly the failure of naloxone to block in their experiments related to the doses of bremazocine and naloxone used, or perhaps that neuroendocrine effect of bremazocine is not mediated by κ-opioid receptor activation. However, both the present neuroendocrine effect of bremazocine and the diuretic effect reported earlier (Leander 1983) were antagonized by naloxone.

Buckingham (1982) demonstrated that morphine and enkephalins stimulated the secretion of corticotrophin-releasing factor (CRF) from rat isolated hypothalamus in-vitro and that these effects were antagonized by naloxone, although the type of opiate receptor involved could not be identified from the data available. The elevation of serum corticosterone by bremazocine that we observed may be an in-vivo consequence of stimulating the release of CRF via the same receptors, and it would be of interest to compare the potency of bremazocine and other selective κ-agonists in stimulating CRF release from the isolated hypothalamus. A possible physiological role of opioid influences on pituitary-adrenocortical function has been considered since the early observation of stimulation by morphine (George & Way 1955), but such a role has not yet been defined.

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