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SHORT COMMUNICATIONS

Ethylketocyclazocine and *N*-cyclopropylmethyl-norazidomorphine are antagonists of morphine-induced analgesia in frog spinal cord

(*Received* 16 *April* 1987; *accepted* 3 *August* 1987)

Ethylketocyclazocine (EKC), a benzomorphan derivative, is a well known agonist of the κ-opioid receptor type in mammalian nervous system [1, 2]. *N*-cyclopropylmethyl-norazidomorphine (CAM) proved to be a strong κ-agonist in rat vas deferens and guinea-pig ileum while having antagonistic effects on μ receptors [3].

Simon *et al.* [4] were the first in establishing a high percentage of benzomorphane (κ/σ) binding site in toad brain, which had a higher affinity to μ-preferring ligands than its mammalian counterpart.

In previous experiments we succeeded in solubilizing the κ-opioid receptor subtype from frog brain [5]. The frog brain differed from the rat brain opioid receptor in that it contained a higher amount of κ-opioid receptor subtype than the rat brain preparation [5, 6]. No literary data on the binding of labelled opioid ligands are available in the spinal cord of frog.

We report here that EKC and CAM behave as antagonists of morphine in pharmacological experiments performed on the decerebrated frog wiping reflex.

Materials and methods

Naloxone hydrochloride was a gift from Endo Laboratories (Garden City, N.Z.) EKC was provided by Sterling Winthrop Research Institute (Rochester NY). CAM was a generous gift of Prof. J. Knoll, Semmelweis University Institute of Pharmacology, Budapest, Hungary. Morphine sulphate was a commercial product.

Analgesia measurements. Frogs (*Rana esculenta*) weighing about 50 g were treated with morphine (20 mg/kg), EKC (20 or 40 mg/kg), naloxone (20 mg/kg) and CAM (10 mg/kg). The drugs were dissolved in 0.7% NaCl solution giving a final drug concentration of 2 mg/ml and injected into the dorsal lymph sac. Control animals were injected with 0.7% NaCl solution. After 20 min the frogs were decapitated and suspended on a hook. Fifteen minutes later the animals were recovering from the spinal shock and the spinal reflex appeared again. One hindleg was immersed into 0.5% acetic acid and the wiping reflex latency time was measured. The same hindlimb was used only after 8–10 min and after several washings. Experiments were repeated three times with both legs registrating six latency times. Three–four animals were used for each experiment.

Results and discussion

The wiping reflex latency time was measured in the decapitated frog after different drug treatments. Morphine had an analgesic action which was antagonized by naloxone in accordance with the data in the literature [7]. However, EKC and CAM have only antagonistic but no agonistic effects in this test (Table 1).

In the present paper we have found that the κ-opioid agonists EKC and CAM behaved as antagonists in the presence of morphine using the frog wiping reflex as an antinociceptive test.

Gillan *et al.* [8] found unexpected antagonism in rat vas deferens by benzomorphans. Using pharmacological and biochemical methods Wood *et al.* [9] reported that EKC and MR 2034 and other benzomorphan derivatives are μ_2 antagonists in rat brain, whereas μ_1 receptors are not affected by them. These are the only pharmacological experiments where an antagonistic effect is described for EKC. CAM behaved as a strong κ-agonist [3] while having antagonist effects in the rat algolytic test similar to naloxone [10]. Our pharmacological experiments on decerebrated frogs showed also an antagonistic effect of EKC on morphine analgesia similar to naloxone, without any antinociceptive effect given alone (see Table 1).

In conclusion, *in vivo* experiments confirmed the antagonistic effect of EKC and CAM of morphine induced analgesia in frog spinal cord. Probably the *N*-cyclopropylmethyl group which is common in the structure of EKC and CAM is responsible for the opioid receptor antagonism.

The results of the *in vitro* binding experiments will be published elsewhere.

Table 1. Foot wiping reflex of frog (*Rana esculenta*)

		Number of experiments	Latency time (sec)
Control	(0.7% NaCl)	16	2.42 ± 1.16
Morphine	20 mg/kg	9	24.4 ± 8.72
EKC	20 mg/kg	14	3.2 ± 1.4
EKC	40 mg/kg	12	4.42 ± 2.87
CAM	10 mg/kg	9	2.33 ± 1.67
Naloxone	20 mg/kg	9	2.2 ± 1.48
Morphine +naloxone	(20 mg/kg) (20 mg/kg)	8	2.6 ± 1.34
Morphine +EKC	(20 mg/kg) (20 mg/kg)	9	3.3 ± 1.5
Morphine +CAM	(20 mg/kg) (10 mg/kg)	9	3.82 ± 2.32

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Reversal of the anti-inflammatory effects of dexamethasone by the glucocorticoid antagonist RU 38486

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The synthetic steroid RU 38486 (11β-(4-dimethyl aminophenyl) 17β-hydroxy, 17α(prop-1-ynyl) estra 4,9-dien-3-one; Mifepristone, RU 486) displays anti-glucocorticoid and antiprogesterone activities *in vivo* and *in vitro* [1, 2]. It has a high affinity for the glucocorticoid receptor *in vitro*, and is a reversible competitive antagonist, with no agonist activity.

The glucocorticoid dexamethasone has potent anti-inflammatory effects in a variety of models. For example, dexamethasone inhibits the formation of inflammatory exudate, the infiltration of leukocytes and release of inflammatory mediators (prostaglandins, leukotrienes and PAF) in the rat carrageenin pleurisy model. Since indomethacin is also active in this model some of the anti-inflammatory effects probably result from the inhibition of prostaglandin synthesis.

In this paper we report the effects of treatment with RU 38486 upon the anti-inflammatory actions of dexamethasone in this model.

Materials and methods

RU 38486 was a generous gift of Roussel-Uclaf, Romainville, France. Dexamethasone (sodium phosphate salt) was obtained from Organon Labs (Cambridge, U.K.) and carrageenin from Sigma (Poole, U.K.).

Pleurisy model

Male Wistar rats (180–220 g) were anaesthetised with ether, and 0.2 ml carrageenin suspension (1% w/v in saline) injected intercostally using a shortened, blunted 21G needle after incision of the skin. Four hours later, the animals were killed, the pleural cavities rinsed with 1 ml heparinised saline and the inflammatory exudate collected. The volume of exudate was estimated by weight, and adjusted to account for recovery of the added 1 ml by deduction of 0.85 ml, the amount recoverable following addition of 1 ml saline to non-treated rats. Leukocytes were counted, and the amount of lyso-PAF present in aliquots of cell-free exudate measured as previously described [3].

Dexamethasone, 50 μg/kg, was given subcutaneously (0.1 ml) at the same time as the carrageenin. RU 38486 was suspended in a vehicle containing 1% carboxymethylcellulose and 0.05% Tween-80, and given orally. Control animals received the vehicle alone.

Results and discussion

Dexamethasone (50 μg/kg) significantly ($P < 0.05$, Student's *t*-test) reduced the exudate volume from 1.04 ± 0.06 to 0.59 ± 0.05 g, the leukocyte infiltration from 74.5 ± 6.1 to 33.5 ± 2.5 × 10^6 cells, and the amount of lyso-PAF (the stable precursor/metabolite of PAF) present in the exudate from 13.4 ± 1.6 to 7.2 ± 1.0 ng (values are mean ± SEM, at least 15 rats, from control and dexamethasone-treated rats, respectively).

Following preliminary experiments RU 38486 was given orally 20 hr and 2 hr before carrageenin and this dosing regime resulted in a dose-dependent inhibition of the effects of dexamethasone (Fig. 1). The ID_{50} was between 20 and

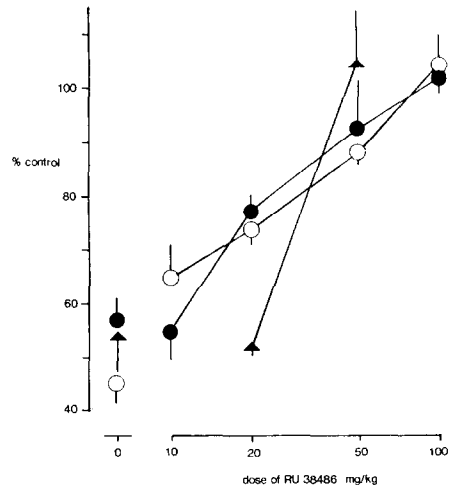

Fig. 1. Anti-glucocorticoid effect of RU 38486. Reversal of the effects of dexamethasone (50 μg/kg) upon leukocyte infiltration (○), exudate volume (●) and lyso-PAF generation (▲) by RU 38486, given as two doses of the amount shown. Each point shows the mean ± SEM from at least 5 rats for each point. The control values (100%) are as in the text, values are shown as % of this control for each parameter measured.