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On the criteria for classifying opiate agonists in rats

FRANK PORRECA, ALAN COWAN*, ROBERT B. RAFFA, RONALD J. TALLARIDA, *Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140, U.S.A.*

It has been postulated that the effects of opioids are mediated through distinct receptor subclasses (Martin et al 1976; Gilbert & Martin 1976). Thus, morphine and ethylketocyclazocine (EK) are prototype agonists at μ and κ receptors, respectively (Martin 1981). Opioids have been classified on the basis of differing sensitivities towards naloxone, dissimilar pharmacological profiles, tolerance and cross-tolerance studies, direct dependence and single-dose suppression studies, and subjective effects in man. Our recent work, however, has emphasized the problems involved in classifying opiate agonists in vivo when data from *several* sources and approaches are considered collectively.

We have compared morphine and EK in rats using two classical opiate endpoints—analgesia and inhibition of gastrointestinal motility; gross behaviour was also observed. Initial analgesic experiments revealed a greater similarity between the prototype μ and κ agonists than might have been anticipated, given the present general acceptance of multiple opiate receptors. For example, the pA_2 value for naloxone (8·1) was the same when obtained with either morphine or EK in the rat tail compression test (Cowan et al 1978) and cross-tolerance could be demonstrated between morphine and EK in the rat tail flick test (Porreca et al 1982). In the present communication, our results on gastrointestinal motility again highlight the similarities, rather than the differences, between morphine and EK.

Male, Sprague-Dawley rats (180–220 g) received morphine sulphate (Mallincrodt) or EK methanesulphonate (Sterling-Winthrop) at 0900 h and 1800 h for 4 days. Twice-daily s.c. doses (expressed as the salt) of 10, 30, 100 and 100 mg kg^{-1} of morphine (Cowan et al 1977) or 5, 10, 20 and 20 mg kg^{-1} of EK, caused tolerance to the inhibitory action of these compounds on gastrointestinal motility. The animals were fasted for 16 h following the last injection, challenged s.c. with morphine, EK or saline and, 20 min later, given a charcoal meal (5 ml kg^{-1}, oral) (Green 1959). A further 25 min later, the rats were killed by cervical dislocation, and the distance travelled by the meal calculated as a percent of the total length of the small intestine. Other groups of rats received equieffective (antimotility) s.c. doses of morphine (7·5 mg kg^{-1}) or EK (10 mg kg^{-1}) 15 min after graded s.c. doses of naloxone hydrochloride (Endo) (75–500 μg kg^{-1}) or saline. Thirty-five min after naloxone, the animals were given the charcoal meal and testing took place a further 25 min later.

In rats pretreated with morphine, neither EK nor morphine had a marked effect on transit. Similar results were obtained when rats, pretreated with EK, were challenged with morphine or EK. The challenge doses (Table 1) were large in relation to our inhibitory A50 values for morphine (1·4 mg kg^{-1}) and EK (2·8 mg kg^{-1}) obtained in the same test but with drug-naive rats (Porreca et al 1981). In other words, we have demonstrated tolerance to the antimotility actions of morphine and EK and, critically, presented evidence of cross-tolerance between these analgesics.

Acute pretreatment with naloxone also failed to differentiate EK from morphine in this procedure since the s.c. A50 values obtained for naloxone against EK (300 μg kg^{-1}) and morphine (133 μg kg^{-1}) differed by a factor of only 2·4.

To allow further interpretation of these results, we investigated 3 other proposed κ agonists—bremazocine, cyclazocine, and nalorphine, the historical κ agonist. High doses of morphine and EK produced behavioural

Table 1. Tolerance and cross-tolerance studies with morphine and ethylketocyclazocine using the charcoal meal test.

Pretreatment[a]	Challenge (mg kg^{-1} s.c.)	n	% Travelled[b] (mean ± s.e.)
Saline	saline	10	40·0 ± 2·7
Saline	morphine (7·5)	5	9·5 ± 5·6***
Morphine	saline	10	43·5 ± 3·3
Morphine	morphine (10)	5	52·8 ± 15·1
Morphine	morphine (30)	5	31·8 ± 3·0*
Morphine	morphine (100)	5	34·6 ± 3·4
Morphine	EK (1)	5	44·2 ± 1·5
Morphine	EK (3)	5	50·5 ± 4·5
Morphine	EK (10)	5	41·6 ± 4·7
Saline	EK (10)	5	5·6 ± 1·2***
EK	saline	10	44·3 ± 1·8
EK	EK (3)	5	59·4 ± 2·9**
EK	EK (10)	5	43·3 ± 8·7
EK	EK (30)	5	38·4 ± 8·8
EK	morphine (3)	5	33·2 ± 9·2
EK	morphine (10)	5	33·3 ± 7·8
EK	morphine (30)	5	9·2 ± 4·8***

[a] Rats received morphine or ethylketocyclazocine (EK) twice daily for 4 days (see text for details) and were challenged as indicated on day 5.
[b] Mean distance travelled by a charcoal meal expressed as a percentage of the total length of the small intestine.
*$P<0·05$, **$P<0·01$, ***$P<0·001$, relative to appropriate saline control (ANOVA, followed by Student's *t*-test).

* Correspondence.

depression in rats; this did not occur with nalorphine, bremazocine and cyclazocine. Furthermore, in contrast to morphine and EK, these agents had poor efficacy in the rat tail flick test and in inhibiting gastrointestinal transit (unpublished results; Green 1959).

Against this background, we have recently learned that EK does not substitute for morphine in rats receiving an i.p. infusion of morphine (Teiger 1974) (Dr M. E. Feigenson, Sterling-Winthrop, personal communication).

Taken together, these data show that the in vivo pharmacological profile of EK, in rats, resembles that of morphine far more than the profiles of other proposed κ compounds such as nalorphine, bremazocine and cyclazocine. The major difference between morphine and EK is the lack of substitution by EK for morphine in Teiger's rat model.

In summary, we call attention to the separation between cross-tolerance and cross-dependence in the rat and pose the following question: what criteria should be used to classify opioids in this species?

Generous samples of ethylketocyclazocine and naloxone were obtained from Sterling-Winthrop and Endo, respectively. The study was supported by Grant DA 02322 from the National Institute on Drug Abuse.

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Muscle fibrosis associated with intramuscular chlorpromazine administration. A preliminary report

* Roger A. Brumback, Larry Empting, Marsha E. Susag, R. Dennis Staton, *Neurology and Psychiatry Services and Neuromuscular Pathology Laboratory, Veterans Administration Medical Center, Departments of Neuroscience and Pediatrics, University of North Dakota School of Medicine, Fargo, North Dakota*

Muscle damage including fibrosis and contracture has been reported as a complication of intramuscular drug administration (Todd 1961; Gray 1967; Hagen 1968; Norman et al 1970; Stark 1970; Mastaglia et al 1971; Aberfeld et al 1968; Steiner et al 1973; Serrano & Wilder 1974). Psychiatric patients frequently receive antipsychotic medication by intramuscular injection. We report a preliminary study of muscle specimens taken from rabbits given intramuscular chlorpromazine and pentazocine showing muscle necrosis and fibrosis, and we compare these findings with the results of pentazocine injection into human muscle.

Methods

Animal study. Twelve New Zealand white rabbits were given daily intramuscular injections in the antero-lateral quadrant of each thigh according to the protocol described below. Injections were given with chlorpromazine (25 mg cc^{-1} solution, Thorazine, Smith-Kline & French Labs), the buffer solution for chlorpromazine (each cc contained ascorbic acid 2 mg, sodium bisulphite 1 mg, sodium sulphite 1 mg, and sodium chloride 6 mg; pH = 4·7), or pentazocine (30 mg cc^{-1} solution, Talwin, Winthrop). Injected doses of chlorpromazine and pentazocine, comparable to human therapeutic doses, were used: 4·3 mg kg^{-1} dose equivalent to 300 mg/70 kg and 1·4 mg kg^{-1} dose equivalent to 100 mg kg^{-1}. Four rabbit thighs were injected with chlorpromazine 4·3 mg kg^{-1} daily for seven days, four thighs were injected with chlorpromazine 1·4 mg kg^{-1} daily for seven days, and four thighs were injected with buffer solution daily for seven days. On day 8 these six rabbits were killed and muscle specimens from the 12 thighs removed for study. Four rabbit thighs were injected with chlorpromazine 1·4 mg kg^{-1} daily for seven days, four thighs were injected with chlorpromazine 4·3 mg kg^{-1} daily for seven days, and four thighs were injected with pentazocine 1·4 mg kg^{-1} daily for seven days. Fourteen days of healing after the last intramuscular injection was allowed before these six rabbits were killed, and muscle from the 12 thighs removed.

At the time of death, the area of the needle injections in each thigh was identified and the skin was removed by dissection to reveal the vastus lateralis muscle underlying the injection site. The muscle was then dissected free and specimens of muscle taken from the injection site were quick-frozen according to standard techniques of Dubowitz & Brooke (1973). Serial sections were stained with a standard histochemical battery (Dubowitz & Brooke 1973).

* Correspondence: VA Medical Center Fargo, North Dakota 58102, U.S.A.