Assessment of physical dependence in rats after continuous intraperitoneal infusion of morphine or ketocyclazocine

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Previous workers have induced physical dependence to morphine in rats by either multiple injection schedules (Hanna, 1960), pellet implantation (Blasig, Herz, Reinhold & Zieglgansberger, 1973) of the use of 'slow-release' emulsions (Laska & Fennessy, 1978). All of these methods involve problems associated with either the determination of the actual dose absorbed or the maintenance of a constant level of exposure to morphine. In the present study an alternative strategy, based on the method described by Teiger (1974), was employed which overcame both of these problems. Using this method dependence was induced by continuous infusion of drug solutions into the peritoneal cavity by means of previously implanted cannulae.

Groups of male rats (Sprague-Dawley, 150–250 g, $n = 6$–7) were used. Morphine (1–100 mg/kg per 24 h) or ketocyclazocine (10–100 mg/kg per 24 h) was continuously infused into the peritoneal cavity (240 μl/h) over a period of 48 hours. At the end of this time abstinence was precipitated by the injection of the opiate antagonist naloxone (3 mg/kg, s.c.). Various signs of abstinence were either measured (weight loss, escape attempts, shaking episodes, writhing episodes) or assessed on an all or none basis (teeth chattering, ptosis, diarrhoea). The degree of weight loss precipitated by naloxone were directly proportional to the dose of morphine or keto-cyclazocine infused. Other signs, such as writhing or shaking, were not linearly related to the dose of analgesic infused but actually declined at higher doses. Martin, Eades, Thompson, Huppler & Gilbert, (1976) have hypothesised that there are distinguishable opiate receptors and suggested that morphine should be considered as the prototype agonist for the μ receptor whilst ketocyclazocine should be considered as the prototype agonist for the k receptor (Martin, *et al.*, 1976). Experiments were conducted to investigate whether keto-cyclazocine could substitute for morphine and suppress the abstinence signs produced in rats abruptly withdrawn from morphine but ketocyclazocine (10–70 mg/kg per 24 h) was without effect in suppressing the signs of abstinence produced in rats during the acute phase of withdrawal from morphine (200 mg/kg per 24 h).

These results indicate that the technique of continuous intraperitoneal infusion provides an improved method of inducing physical dependence to opiate drugs in rats. By careful observation of the signs of abstinence precipitated after naloxone challenge the degree of dependence can be related directly to the dose of opiate administered. Results of cross suppression tests using morphine and ketocyclazocine indicate a distinction between analgesics which interact with the so-called μ and k receptors.

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Dependence, tolerance and cross-tolerance induced by morphine and ethylketocyclazocine

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The proposed k-agonist ethylketocyclazocine neither precipitates nor suppresses the abstinence syndrome in morphine-dependent monkeys (Villarreal & Seevers, 1972).

Mice and guinea-pigs were pretreated with a single subcutaneous injection of a slow-release emulsion (Collier, Francis & Schneider, 1972) containing morphine (300 mg/kg) or ethylketocyclazocine (30 mg/kg). The animals were allowed between 2 and 96 h of exposure to the emulsion prior to testing.

Tolerance in mice was assessed *in vivo* by challenging pre-treated animals with standard doses of agonists (morphine, 40 mg/kg; ethylketocyclazocine,

5 mg/kg; i.p.) and measuring hot plate reaction times and respiratory rates at peak effect. Dependence was measured by challenging pretreated mice with naloxone (1 mg/kg). Hot plate reaction times and respiratory rates were measured for one hour following injection. Frequency of jumping, 'wet dog' shakes, shivering, weight loss, and other signs of withdrawal were also noted.

The increase in hot plate reaction times and depression of respiratory rates in animals pretreated with either morphine or ethylketocyclazocine for between 12 and 72 h prior to acute administration of either morphine or ethylketocyclazocine, were significantly lower than in control animals pretreated with vehicle. The degree of dependence was difficult to assess, but similar signs of withdrawal following administration of naloxone were seen in animals pretreated with either morphine of ethylketocyclazocine.

Pieces of guinea-pig ileum were set up for transmural stimulation (0.1 Hz, 0.5ms duration, supramaximal voltage). Tolerance was assessed by a comparison of the ID_{30} agonist dose to inhibit twitch height in pretreated animals with the ID_{30} agonist dose derived from equivalent controls. Degree of dependence was assessed using a method similar to that described by Schultz & Herz (1976) in which segments of ileum from pretreated guinea-pigs contract when challenged with naloxone.

Tolerance, cross-tolerance, and dependence was seen in animals treated with morphine or ethylketocyclazocine for 6–96 hours.

S.J.W. is an S.R.C. student.

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Characterization of opiate receptors in the isolated rat rectum

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The isolated rectum of the rat has been reported to contract in response to low concentrations of several opioid peptides (Nijkamp & van Ree, 1978). In the present study a field stimulated rat rectum preparation was developed in which the electrically-evoked contractions were shown to be inhibited by both the classical opiate normorphine, and the opioid peptides leucine-and methionine-enkephalin and β-endorphin.

Segments of rat rectum were induced to contract by applying trains of pulses (1 ms pulses at 2 pulses/s for 2 s every 40 s) by means of ring electrodes above and below the tissue. The contractions so induced were biphasic, and the first component was inhibited by opiates (IC_{50} for leu-enkephalin = 10.8 ± 2.67 nM)

Table 1 Comparison of agonist potency and reversibility by naloxone of representative opiates in three *in vitro* preparations

	Guinea Pig Ileum[a]		*Mouse Vas Deferens*[a]		*Rat Rectum*	
	Potency[b]	*Ke naloxone* (nM)	*Potency*[b]	*Ke naloxone* (nM)	*Potency*[b]	*Ke naloxone* (nM)
Leu-enkephalin	1	1.74	1	21.4	1	21.7 ± 2.34
Met-enkephalin	2.1	1.94	0.28	28.3	1.98 ± 0.33	19.7 ± 1.76
β-endorphin	8.9	2.53	0.138	30.5	0.198 ± 0.037	28.8[c]
Normorphine	4.52	1.83	0.07	4.75	0.141 ± 0.038	3.68 ± 1.01

[a] Data adapted from Shaw & Turnbull (1978).
[b] Potencies are expressed as ratios to the IC_{50} of leu-enkephalin.
[c] Mean of 2 observations. All other figures represent the mean of at least 5 determinations.