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A DOSE-RATIO COMPARISON OF MU AND KAPPA AGONISTS IN FORMALIN AND THERMAL PAIN

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Summary

The effects of putative mu and kappa agonists, with and without naloxone, were compared in the formalin and tail flick tests in rats. The mu agonist sufentanil was more potent in the tail flick test than the formalin test while the opposite was true for the kappa agonist ethylketocyclazocine (EKC). MR2034 was equipotent in the two tests and in the tail flick test, analgesia decreased at high doses. The naloxone (0.1mg/kg) dose-ratios (DR) for sufentanil and EKC were 3 to 7 times larger for the tail flick test than the formalin test. From this and other DR studies it is argued that in thermal pain tests, opioid analgesia is mediated primarily by mu receptors while in non thermal tests kappa effects predominate.

It is generally accepted that there are several types of opioid receptors (9,16,33) but the contributions of these receptors to the analgesic effect of opioids is not well established. There is considerable evidence that analgesia is induced by both mu and kappa agonists (6,12,22,25,26,28,32). but the role of kappa receptors in analgesia is uncertain. It has been reported that the pA_2 for naloxone is the same with the kappa agonist ethylketocyclazocine (EKC) as with the mu agonist morphine (6,32) and it has been argued that analgesia with putative kappa agonists involves a mu receptor(20). On the other hand some investigators have failed to find analgesia with putative kappa agonists (27,31) and others have reported that naloxone is a more potent antagonist of morphine analgesia than analgesia induced by EKC or dynorphin (21,26). Recently it has been suggested that kappa agonists may be more potent analgesics against some pain stimuli than others. Specifically it has been proposed that mu receptors are primarily involved in analgesia in tests employing a cutaneous heat stimulus whereas kappa receptors may be involved in analgesia of pain produced by other stimuli such as peritoneal irritants (31).

The present investigation explored the role of mu and kappa receptors in different types of pain by comparing the analgesic effects and naloxone sensitivity of opioid agonists in a thermal pain test, the tail flick test, and a non- thermal test, the formalin test (7), which assesses an animals response to a minor tissue injury produced by a subcutaneous injection of dilute formalin.

Materials and Methods.

Animals and General Procedure Five or 6 male Long Evans hooded rats (200 - 250g) were assigned to be tested at each dose of the opioid agonists alone or in combination with naloxone. Because tolerance to morphine develops at different rates in the tail flick test and formalin test (1) each rat was tested once only. The time of the peak effect of each agonist was determined by examination of the time course of a midrange dose in the

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tail flick test. Naloxone (0.1mg/kg) was injected 15 min before the expected peak effect of the agonists so that its peak effect would correspond with that of the agonists(29). For morphine the mean time of peak effect was 30 min thought there was considerable variability. In other experiments the peak effect has been found to be at 60 min (14). Because of the uncertainty concerning the true time of peak effect naloxone dose ratios were established at 30 and 60 min after morphine.

Drugs Sufentanil citrate (gift of Janssen Pharmaceutica, Beerse, Belgium), ethyketocyclazocine methanesulfonate (gift of Sterling-Winthrop Research Institute, Renselaer, New York) and MR2034 free base (gift of Boehringer Ingelheim (Canada) Ltd.) and naloxone hydrochloride (gift of Endo Laboratories, Garden City, New York) were dissolved in isotonic saline. All drugs were injected s.c in a volume of 1 ml/kg except doses of MR2034 above 15 mg/kg and EKC above 5mg/kg which were injected as solutions of 15 mg/ml and 5 mg/ml respectively.

Tail Flick Test Responses to thermal pain were assessed by the latency with which a rat removed its tail from 55 degree C water. During testing rats were restrained in wire restraining tubes which they entered approximately 10 min before the first drug injection. The tail was marked 5cm from the tip and at each test a beaker of hot water was applied to the tail up to the 5cm mark. If the rat did not remove its tail from the water in 10 seconds the test was terminated to minimize tissue damage.

Formalin Test A detailed description of this test and its rationale can be found in Dubuisson & Dennis (7). Briefly, .05 ml of 2.5% formalin was injected s.c. into the plantar surface of one of a rat's hindpaws. The rat was then placed in a $30cm^3$ plexiglass chamber which allowed an unobstructed view of the rat and, via an inclined mirror, its ventral surface and feet. Pain was rated by recording the amount of time the rat stood or walked firmly on the injured paw (Pain rating =0), favoured the paw (Pain Rating=1), elevated the paw with at most the nails touching the floor (Pain Rating=2), or licked and chewed at the paw (Pain Rating = 3). The pain score was calculated for ten min periods as 1/600 x the sum across rating categories of the time spent in each category (secs) multiplied by the Pain Rating.

Formalin pain is high immediately after formalin is injected, falls to a low point 15 to 20 min later then rises again to a high level which remains steady for 30 to 40 min (7). In this experiment drug injections were timed so that peak effects would occur 40 min after formalin injection and dose effect curves were calculated from pain ratings accumulated over the period 35 to 45 min after formalin injection.

Analysis of Data Pain scores were converted to percent of the maximum possible effect by the formula

$$\%MPE = \frac{\text{(pain score under drug - control score)}}{\text{(maximum possible analgesia score - control score)}} \times 100$$

For the tail flick test the control score was 2.5 sec and the maximum score 10 sec. For the formalin test the control score was 2.2 and the pain score representing maximum analgesia was 0. The control scores represent the long run averages of baseline pain scores in this laboratory. For the formalin test the control score has a standard error approximately .01 for a group of six rats.

Mean analgesia scores were plotted against log dose and a straight line was fitted to the steeply rising portion of the dose effect curves by the method of least squares. An MPE_{50} was defined as the dose at the half maximal effect. A statistical estimate of the MPE_{50} and its standard error was calculated from the data for individual animals by jacknifing the regression lines and interpolated MPE_{50}s (17,23). Jacknifing is a method of directly assessing variability of statistics which offers ways to set sensible confidence limits in complex situations. It is an iterative procedure which computes pseudo-values for statistics from all possible subsets of n-1 of the data points. The mean and variance