Short communication

INTRATHECAL MORPHINE SLOWS GASTROINTESTINAL TRANSIT IN RATS

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Intrathecal (i.th.) (by direct lumbar puncture) and intraperitoneal (i.p.) administration of morphine (30–100 μg/rat) caused a dose-related inhibition of gastrointestinal transit in the rat. Pretreatment with i.th. naloxone (5 μg at -5 min) reversed the effects of i.th., but not i.p., morphine. These results suggest that the spinal cord appears to be a target site for the inhibitory effects of morphine on gastrointestinal transit in the rat.

Gastrointestinal transit   Intrathecal   Morphine   Naloxone   Rats

1. Introduction

The brain (Burks, 1978) and spinal cord (Porreca and Burks, 1983) have been recognized as independent target sites for centrally acting drugs which influence gastrointestinal function. Central, as well as peripheral, administration of opioids can inhibit gastrointestinal transit (Green, 1959; Stewart et al., 1978). Intrathecal (i.th.) injection of morphine in mice, by direct lumbar puncture, results in a dose-dependent, naloxone-reversible inhibition of gastrointestinal transit (Porreca et al., 1983). Surprisingly, however, i.th. administration of morphine to rats was not effective in slowing gastrointestinal transit, suggesting the existence of a species difference between mice and rats in this endpoint (Vaught et al., 1983). In the latter study, the drug was delivered to the spinal subarachnoid space by means of a surgically implanted cannula as described by Yaksh and Rudy (1976). Results

using this technique have occasionally been difficult to explain. For example, Herman and Goldstein (1985), using the i.th. cannula technique, reported that dynorphin A is a more potent analgesic when given within 1 day of i.th. cannulation as opposed to 7 days post-surgery. Thus, it is possible that the method of drug delivery (i.e. i.th. cannula vs. direct lumbar puncture) is responsible for the divergent results seen with the morphine antitransit effects in mice and rats. In the present work, we determined whether i.th. morphine, delivered by direct lumbar puncture to rats could affect gastrointestinal transit. Additionally, the possibility of i.th. morphine leaking out of the spinal subarachnoid space and affecting the gut at peripheral sites was examined using i.th. naloxone. We now report that morphine is active at spinal sites in slowing gut transit in the rat when it is given by direct lumbar puncture.

2. Materials and methods

Male, Sprague Dawley rats (Charles River, 60-80 g) were starved overnight prior to experimentation.

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Water was available ad libitum. Testing consisted of i.th. pretreatment with either saline or naloxone (5 μg/rat) (Sigma) given 5 min prior to the injection of test compound (morphine or saline). The test compound was given by either i.th. or i.p. routes; i.th. injections were made by direct lumbar puncture between the fifth and sixth lumbar vertebrae in unanesthetised rats as described by Hylden and Wilcox (1980). A constant 5 μl volume was employed and delivered using a Hamilton microliter syringe fitted with a 30 gauge needle. I.p. injections were made using a constant 0.5 ml volume. The response at each dose was determined using 8-12 rats.

Gastrointestinal transit was measured by the method described by Porreca and Burks (1983). Immediately following injection of test compound, rats received an oral injection of ^{51}Cr as sodium chromate in saline (0.5 μCi, 0.2 ml/rat) (New England Nuclear). Thirty-five minutes after administration of ^{51}Cr, the animal was killed by cervical dislocation, and the stomach and small intestine removed. The small bowel was then placed on a ruled template and divided into 10 segments of equal length. Each of the 10 pieces, plus the stomach, were placed into individual and consecutive vials. Radioactivity was determined for each vial by gamma counting for 1 min. Transit was calculated by the geometric center method (Miller et al., 1980). Values range from 1.0 to 10, with a lower numeric value indicating slower transit of the radioactive marker. Transit data were subjected to analysis of variance, and where significance was indicated, followed by Student's t-test for unpaired data.

3. Results

I.th. administration of morphine (30-100 μg/rat) had a marked dose-related inhibitory effect on gastrointestinal transit (fig. 1). An examination of possible peripheral gut effects of morphine by i.p. drug administration showed a significant, but less pronounced, effect over a similar dose range (fig. 2). In order to determine if the effects of i.th. morphine were occurring at the spinal level, the experiments were repeated in
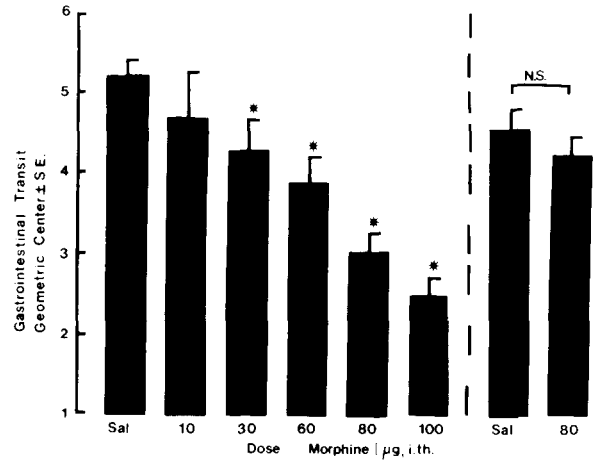

Fig. 1. Dose-response relationship for i.th. morphine inhibition of gastrointestinal transit in the rat. Graded doses of morphine were given concurrently with oral sodium chromate/saline and transit was measured at +35 min. The effect of i.th. naloxone (5 μg at −5 min) is shown in the 2 bars on the right.

animals pretreated with naloxone (5 μg at −5 min). I.th. naloxone blocked the inhibitory transit effects of a subthreshold dose of i.th. morphine (80 μg/rat) (fig. 1), whereas the same i.p. morphine dose (80 μg/rat) was still effective in slowing gut transit (fig. 2) in naloxone pretreated animals.
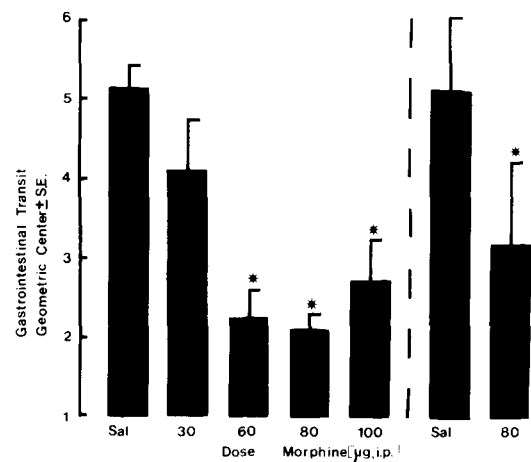

Fig. 2. Dose-response relationship for i.p. morphine inhibition of gastrointestinal transit in the rat. Graded doses of morphine were given 20 min prior to oral sodium chromate/saline and transit measured after a further 35 min. The effect of i.th. naloxone (5 μg at −5 min) is shown in the 2 bars on the right.

4. Discussion

Opioids can affect gastrointestinal transit by both central and peripheral sites of action (Green, 1959; Stewart et al., 1978). Porreca et al. (1983) demonstrated that morphine injected i.th. in mice caused inhibition of gut transit, a result confirmed by Vaught et al. (1983). Morphine can, therefore, act independently at spinal or supraspinal sites to slow gastrointestinal transit in the mouse (Porreca and Burks, 1983). Our previous results had indicated that while i.th. morphine in rats caused analgesia, antitransit effects could not be demonstrated (Vaught et al., 1983). This result was surprising in light of the results in the mouse, as well as the effectiveness of i.th. bombesin in slowing gut transit when delivered by the same technique (Gmerek et al., 1983).

Herman and Goldstein (1985) have recently demonstrated that chronic i.th. cannulation using the Yaksh and Rudy technique (1976) can affect the analgesic potency of opioid peptides such as dynorphin A. With analgesia as the endpoint, dynorphin A was found to be equipotent to morphine when drug was injected within 1 day after cannula implantation. Seven days after surgery, however, dynorphin was an order of magnitude less potent than morphine (Herman and Goldstein, 1985). This study, together with the antitransit effectiveness of i.th. morphine given by direct lumbar puncture in the mouse, suggested that the method of drug delivery might be responsible for the lack of observable gut effects in our previous study employing the cannula technique for delivery of opioids (Vaught et al., 1983). Alternatively, the dose delivered in the previous work, while effective in producing analgesia, may not have been high enough in relation to the time following cannulation of the animals. A further possible difference between the previous and present results may relate to the method employed for measuring transit. Vaught et al. (1983) used a charcoal meal technique (Green, 1959) while the present work employed a radiolabelled, liquid marker. While evidence exists regarding differential emptying of solids and liquids, this possibility seems remote in this case, as i.th. morphine by lumbar puncture is effective in slowing transit in the mouse when transit is

evaluated by either technique (unpublished results). In any case, results from the present study demonstrate unequivocally that, using a different means of i.th. drug delivery (direct lumbar puncture) morphine does inhibit gastrointestinal transit in rats. Morphine, given peripherally was also effective of the same dose range (30-100 μg/rat).

In order to determine if i.th. morphine was acting centrally or peripherally, rats were pretreated with i.th. naloxone. Pretreatment with this antagonist eliminated the antitransit effects of 80 μg/rat of i.th., but not i.p., morphine. These data suggest, therefore, that i.th. morphine acts at spinal sites to initiate inhibition of gut transit in rats. In support of this finding, it should be noted that intracerebroventricular morphine also slows gut transit over exactly the same dose range (Porreca et al., 1982).

In summary, we have demonstrated that i.th. morphine inhibits gastrointestinal transit in a dose-related fashion in rats. This effect is prevented by i.th. pretreatment with naloxone. Therefore, gut transit is slowed in both mice and rats in response to i.th. morphine. The reasons for the ineffectiveness of i.th. morphine after delivery by chronically implanted cannula are unclear. Surprisingly, this difference in effectiveness between the 2 methods of drug delivery does not apply to bombesin, a peptide which also slows gut transit, as this agent is effective in rats by either technique (Gmerek et al., 1983; Kosla and Porreca, unpublished observations). Further experimentation will be necessary to determine if species differences exist with other opiates or opioid peptides.

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