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Neostigmine antagonism of morphine's effects on intestinal transit†

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Morphine inhibits gastrointestinal transit more effectively if given intracerebroventricularly rather than by a peripheral route (Parolaro et al 1977; Stewart et al 1978; Schulz et al 1979). The inhibition of intestinal transit after centrally administered morphine is completely abolished by opiate antagonist administered intracerebroventricularly or by transection of the vagus nerve (Stewart et al 1978). The inhibition of intestinal transit produced by systemically administered morphine is also abolished by opiate receptor blockade but not by transection of the vagus (Parolaro et al 1977; Stewart et al 1978). If, as these data suggest, the effects of centrally and systemically administered morphine involve different mechanisms, then it should be possible to antagonize each effect selectively with pharmacological agents. To test this hypothesis, the intestinal effects of morphine given centrally or systemically were assessed after the administration of neostigmine or atropine methylbromide.

Materials and methods

Adult male rats (Sprague Dawley, U.S.A.), 200–250 g, were implanted with indwelling silicone catheters in the proximal small intestine (Stewart et al 1978). Each animal was anaesthetized with sodium pentobarbitone (50 mg kg^{-1} i.p.) and the catheter was anchored in the lumen of the proximal duodenum, approximately 3 cm distal to the gastroduodenal junction. The free end of the catheter was led subcutaneously from an abdominal stab wound to the mid-scapular region, where it was brought to the outside of the animal through a cutaneous puncture wound. The free end of the catheter was wrapped in gauze and protected by a shoulder harness fashioned from several strips of paper tape.

A number of animals were additionally implanted with a polyethylene cannula (PE 10) in the right lateral cerebral ventricle (Robinson et al 1969; de Balbian Verster et al 1971). The cannula was introduced into the skull to a depth of 4 mm through a hole made 1·5 mm lateral and caudal to the bregma. A second hole was made in the skull close to the cannula entrance for a small stainless steel anchoring screw. The cannula was secured to the skull and the anchoring screw with a mound of dental acrylic. The head wound was closed with wound clips.

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Intestinal transit was determined by instilling 0·2 ml of $Na_2{}^{51}CrO_4$ (0·5 μCi) into the duodenal catheter. The instillation of the chromium solution was followed by instillation of an additional 0·5 ml of 0·9% NaCl (saline) to flush the radioactive marker from the catheter into the intestinal lumen. The animals were killed by cervical dislocation 10 min after chromium administration. The small intestine was then carefully dissected after metal clamps were applied at the gastroduodenal and ileocecal junctions. The small intestine was placed on a ruled template where it was divided into ten equal segments. The individual segments were placed consecutively in counting vials and the gamma emissions were recorded for a 1 min period using a Beckman gamma counter (Biogamma).

Calculation of intestinal transit

Intestinal transit was calculated for each animal as follows: the percentage of total radioactivity in the whole small intestine was determined for each of the 10 individual intestinal segments. The percentage data obtained were transformed to cumulative percent radioactivity for each intestinal segment (Stewart et al 1978). The plot of cumulative percent radioactivity *vs* intestinal segment from segment 1 (proximal duodenum) to segment 10 (terminal ileum) results in a linear plot with a negative slope. The x-intercept of the resulting plot, calculated from the regression equation and expressed as a percentage of the small intestine, quantifies the maximum percentage of small intestine traversed by chromium. Treatment group x-intercept values for each experiment were analysed statistically using an analysis of variance. Individual differences between treatments were determined with a Tukey's test (Steel & Torrie 1960). Probability values equal to, or less than, 0·05 were considered significantly different.

Experimental protocol

The animals were housed singly in cages with wire mesh bottoms. Experiments were performed on the unanaesthetized rats 3 to 5 days after surgical preparation. All animals were fasted for 18 h before experimentation. Water was freely available.

Morphine sulphate or saline was administered either by subcutaneous (s.c.) or intracerebroventricular (i.c.v.) injection 30 min before intraduodenal instillation of chromium. Morphine was administered at a dose of 5 mg kg^{-1} s.c. or 30 μg (total dose) i.c.v. All s.c. injections were given in a volume of 1 ml kg^{-1}. All i.c.v. injections were given in a total volume of 10 μl. The animals were pretreated with either atropine methylbromide (6 mg kg^{-1} s.c.), neostig-

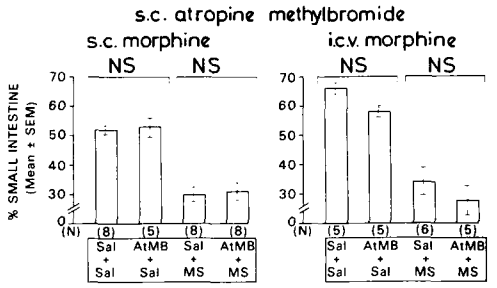

FIG. 1. Percentage (mean ± s.e.m.) of small intestine intestine traversed by chromium after s.c. (5 mg kg^{-1}) or i.c.v. (30 μg) morphine (MS) or saline (Sal) in animals pretreated with atropine methylbromide (AtMB, 6 mg kg^{-1} s.c.) or saline. Responses overscored by the same line are not significantly different by a Tukey's test. AtMB did not alter normal (Sal + Sal) intestinal transit and did not affect the intestinal antipropulsive actions of MS given s.c. or i.c.v. NS = not significant, $P > 0{\cdot}05$; (n) = no. of animals in each group.

mine methylsulphate (0·1 mg kg^{-1} s.c.) or saline (1 ml kg^{-1} s.c.) 30 min before injection of morphine or saline. The doses of atropine methylbromide and neostigmine have been demonstrated previously to provide cholinergic blockade or activation without affecting normal rat intestinal transit (Ruwart et al 1979).

Drugs employed were atropine methylbromide (Sigma Chemical Co, USA), morphine sulphate (Merck and Co, USA), and neostigmine methylsulphate (Prostigmin, Roche Laboratories, USA). Doses of all drugs are expressed as their salts.

Results

Effects of atropine methylbromide on morphine

Atropine methylbromide (6 mg kg^{-1}) or saline (1 ml kg^{-1}) was administered subcutaneously 30 min before s.c. morphine (5 mg kg^{-1}) or saline (Fig. 1, left). Peripheral atropine methylbromide followed by s.c. saline did not alter intestinal transit when compared with results from animals given two injections of saline. Peripheral morphine significantly inhibited intestinal transit when given after saline. Pretreatment with s.c. atropine methylbromide did not alter the intestinal antipropulsive effect of peripheral morphine.

In a separate experiment, atropine methylbromide (6 mg kg^{-1}) or saline (1 ml kg^{-1}) was administered subcutaneously 30 min before i.c.v. morphine (30 μg, total dose) or saline (10 μl). Peripheral atropine methylbromide followed by i.c.v. saline did not significantly alter intestinal transit when compared with results from animals given two injections of saline (Fig. 1, right). Central morphine significantly inhibited intestinal transit when given after s.c. saline. Pretreatment with s.c. atropine methylbromide did not alter the intestinal antipropulsive action of central morphine.

Effects of neostigmine on morphine

Neostigmine (0·1 mg kg^{-1}) or saline (1 ml kg^{-1}) was administered s.c. 30 min before morphine (5 mg kg^{-1} s.c.) or saline (Fig. 2, left). Peripheral administration of neostigmine followed by s.c. saline did not significantly affect intestinal transit when compared with results from animals given two injections of saline. Peripheral morphine significantly inhibited intestinal transit when given after saline. Pretreatment with peripheral neostigmine successfully antagonized the intestinal antipropulsive effect of peripheral morphine. Atropine methylbromide (6 mg kg^{-1}) given subcutaneously 30 min before peripheral neostigmine restored the intestinal antipropulsive action of peripheral morphine.

Neostigmine (0·1 mg kg^{-1}) or saline (1 ml kg^{-1}) was also administered s.c. 30 min before i.c.v. morphine (30 μg, total dose) or saline (10 μl) in a separate experiment. Peripheral administration of neostigmine followed by i.c.v. saline did not significantly alter intestinal transit when compared with results from animals given two injections of saline (Fig. 2, right). Central morphine significantly inhibited intestinal transit when given before central saline. Pretreatment with s.c. neostigmine did not affect the intestinal antipropulsive action of central morphine.

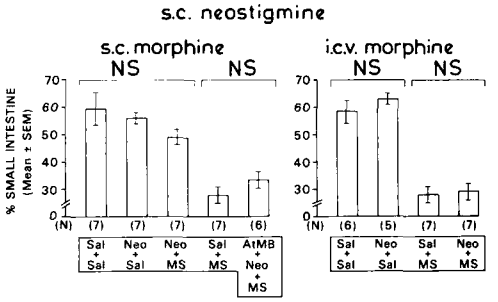

FIG. 2. Effects of s.c. (5 mg kg^{-1}) or i.c.v. (30 μg) morphine (MS) or saline (Sal) in animals pretreated with neostigmine (Neo), 0·1 mg kg^{-1} s.c.) or saline. Neo antagonized the effects of s.c. but not i.c.v. MS. Atropine methylbromide (AtMB, 6 mg kg^{-1} s.c.) reversed the antagonism of s.c. MS by Neo. See Fig. 1. NS = not significant, $P > 0{\cdot}05$; (n) = no. of animals in each group.

Discussion

Rat intestinal transit was determined by measuring the progression of an intraduodenally administered radioactive marker after central or peripheral administration of morphine. Morphine produced nearly equal inhibition of intestinal transit when given intracerebroventricularly at a total dose that was approximately thirty times less than the average total dose given subcutaneously. The results are in agreement with previous studies (Parolaro et al 1977; Stewart et al 1978; Schulz et al 1979).

The inhibitory effects of central and peripheral morphine on intestinal transit were tested separately after peripheral administration of either atropine methylbromide or neostigmine, drugs which inhibit or enhance, respectively, the

effects of cholinergic nerve activation. Both atropine methylbromide and neostigmine resist penetration into the brain (Irwin & Hein 1962; Witter et al 1973); thus, the actions of both agents were probably confined mainly to peripheral structures. The present results confirm an earlier study by Ruwart et al (1979) which showed that neither atropine nor neostigmine at the doses employed influence normal rat intestinal transit. In the present study, atropine methylbromide did not affect the inhibition of rat intestinal transit after either central or peripheral administration of morphine. Enhanced cholinergic transmission by neostigmine, however, antagonized the intestinal effect of peripheral, but not central morphine. To determine whether this antagonism resulted from activation of muscarinic cholinergic mechanisms, animals were treated with atropine methylbromide before administration of neostigmine and morphine. Atropine methylbromide reversed the neostigmine antagonism of systemically administered morphine. This result suggests that specific activation of muscarinic cholinergic mechanisms in the periphery was responsible for the selective antagonism of peripherally administered morphine.

The finding that neither enhancement nor inhibition of peripheral cholinergic mechanisms influenced the intestinal response to central morphine was particularly surprising. Since inhibition of rat intestinal transit after central morphine administration is abolished by transection of the vagus nerve (Stewart et al 1978), the peripheral conducting pathway in the vagus may be non-cholinergic. Schulz et al (1979) failed to alter the intestinal inhibitory response to central morphine with peripheral injection of quaternary naloxone. These authors concluded that the peripheral conducting pathway did not include an opiate receptor outside of the brain. Thus, the nature of the vagal pathway mediating the intestinal response to central morphine is still uncertain.

Unlike the actions of central morphine, the actions of peripherally administered morphine on rat intestinal transit were antagonized by neostigmine. The selective antagonism of peripheral morphine by a drug that enhances the actions of neuronally released acetylcholine, provides further evidence that central and peripheral morphine inhibit intestinal transit in the rat by different mechanisms. Apparently, the local inhibitory action of morphine on the gastrointestinal tract is antagonized by enhanced muscarinic cholinergic activity.

Morphine inhibits electrically evoked contractions of guinea-pig isolated ileum by decreasing the release of acetylcholine from myenteric neurons (Schaumann 1957). Subcutaneous morphine also inhibits contractions recorded from the small intestine of the conscious rat (Weisbrodt et al 1980). Neostigmine might inhibit the effects of morphine on intestinal contractile activity. Alternatively, since morphine increases water and electrolyte absorption from the small intestine (Mailman 1980), neostigmine might affect the actions of morphine on intestinal water and electrolyte absorption. Studies that monitor intestinal smooth muscle contractions and/or measure water absorption from the intestine may help explain the antagonism of peripheral morphine by neostigmine.

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