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The Effect of Acute and Chronic Administration of Morphine and Morphine Withdrawal on Intestinal Transit Time in the Rat

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Abstract—The effects of acute and chronic morphine administration and of morphine-withdrawal on intestinal transit time of a liquid meal were investigated using rats. Many experiments have assessed the effects of acute morphine administration on intestinal transit, but the intestinal effects of chronic morphine administration have been neglected. Our results showed no significant differences between morphine-dependent and control animals when assessing the leading edge of the liquid meal infusion, its distribution and geometric centre (G.C.). However, during naloxone-precipitated withdrawal from morphine, the leading edge of the infusion and its G.C. were significantly distal to values obtained from other groups. Acute morphine administration caused delayed intestinal transit of a meal infusion, an effect partly caused by significant retention of the infusion in the stomach and duodenum. The leading edge of the meal infusion and G.C. were significantly proximal to values obtained from other groups of animals. The results show that morphine-dependent rats develop complete tolerance to the delayed intestinal transit of a meal observed after acute morphine administration and that withdrawal from morphine accelerates intestinal transit of a liquid meal.

It has been assumed that the diarrhoea resulting from opiate withdrawal is caused by an increase in gastrointestinal motility. This is an assumption based largely on the fact that segments of intestine removed from morphine-dependent animals display excessive spontaneous activity when suspended in morphine-free solution and actively contract when exposed to opioid antagonists (Kaymakcalan & Temelli 1964; Schulz & Herz 1976; Gintzler 1979; Huidobro-Toro & Way 1981). Such antagonist precipitated withdrawal is associated with the release of a range of neurotransmitters such as acetylcholine, vasoactive intestinal polypeptide, substance P and 5-hydroxytryptamine (5-HT) (Gintzler 1979, 1980; Collier et al 1980; Huidobro-Toro & Way 1981; Chahl 1986). The release of these substances could cause the observed muscle contractility, but whether it is transferred into propulsion of contents along the entire length of the intestine has not as yet been determined. The only in-vivo study to date that has attempted to answer this problem has relied on the indirect measurement of muscle contractility as detected by changes in the migrating myoelectric complex and the number of spike potentials. These parameters were shown to be disrupted during naloxone-induced withdrawal in conscious rats. However, recordings were only monitored from four points along the length of the small intestine (Kuperman et al 1987). The dramatic effect of opiate withdrawal on the intestine implies that the tissue becomes tolerant to morphine administration.

Surprisingly, there are only a few reports showing that this does occur. For instance, it has been established that tolerance develops to the contractile effect of morphine in the dog small intestine in-vitro (Burks & Grubb 1974), an effect mediated by endogenous 5-HT (Burks 1973). Two similar studies have shown that administration of morphine for seven days to conscious rats produces incomplete tolerance to the morphine-induced reduction in transit (Burks et al 1976; Weisbrodt et al 1977). However, withdrawal diarrhoea can be provoked in rats after only one or two days of morphine exposure (Collier et al 1972). Although transit or motility parameters were not measured, this may indicate very rapid induction of tolerance. Consequently, the aims of the present study were to determine (i) the effects of morphine withdrawal on intestinal transit and (ii) whether rapid tolerance occurs to the antitransit effect of morphine.

Changes in intestinal transit have been assessed by measuring the distribution of a radioactively labelled nutrient solution infused at physiological rate into the duodenum of conscious rats. This method has the advantage over in-vitro and myoelectric methods in that it measures the functional effect of morphine treatment along the entire length of the intestine and it measures the effects in fed rather than fasting motility.

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Methods

Animals

Experiments were carried out on a total of 29 adult male albino rats equipped with chronic duodenal cannulae. Animals were obtained from Sheffield field laboratories and weighed between 250 and 300 g. Postoperatively, animals were housed singly on wire-bottomed mesh cages and allowed free access to food and water. 36 h before the experiment commenced solid food was withdrawn from all animals.

Surgical procedure

A plastic cannula (Silastic ID 0·51 mm, OD 0·94 mm, Dow Corning), 25 cm in length, was implanted in the duodenum of all animals. This procedure was performed using pentobarbitone (60 mg kg^{-1} i.p.) anaesthesia. The abdomen was opened via a midline incision and the cannula placed in the duodenal lumen approximately 2–3 cm distal to the gastroduodenal junction via a stab wound. The intestinal wound was closed by tying a purse-string suture around the cannula making sure the lumen of the cannula was not occluded. Sufficient cannula was left free in the abdominal cavity to allow the gastrointestinal tract full mobility. The abdominal incision was closed in two separate layers, the muscle and then the skin using a sterile braided silk suture 5-0 (Mersilk, Ethicon). About 2–3 cm from the end of the cannula a small square of nylon mesh was secured using silicone glue (Medical adhesive type A, Dow Corning). The cannula was tunnelled subcutaneously from the duodenum to the midscapular region where it was exteriorized via a cutaneous puncture, the piece of nylon mesh lying under the skin forming an anchorage point for the cannula. Initially three stitches were used to secure the cannula in position and the exposed end was plugged with a blunt-ended pin. Each rat was allowed a post-operative recovery period of one week before any experimental procedures were performed. A small volume of saline was infused every day into the duodenum of each rat to ensure the cannula remained patent.

Experimental protocol

The experiments were performed over a three day period. The animals were divided into five groups. One group of five animals was used to measure the effect of an acute dose of morphine (10 mg kg^{-1} s.c. in saline). Two groups of six animals received a s.c. injection of morphine in a depot emulsion (Warhurst et al 1984) into the back of the neck; a further two groups of six animals received emulsion without morphine. The emulsion contained 300 mg morphine HCl 10 mL^{-1} and the dose given to induce physical dependence was 300 mg kg^{-1} over a 48 h period. This was administered as three divided s.c. injections, 25% of the dose on both the morning and afternoon of the first day, and 50% in the morning of the second day. The rats are then used for the transit studies on the third day.

All animals were infused intraduodenally for 30 min with a 10% solution of Vitalife in water, at a rate of 2 mL h^{-1}. Vitalife is a balanced food preparation based on dried skimmed milk with added vitamins and minerals. The Vitalife was radiolabelled with ^{99}technetium (Tc) sulphur colloid (liquid phase) to give an activity of 25 μCi (0·93 mBq) in each 3 mL aliquot to be infused into the animal. After infusion for 30 min, control and morphine-dependent animals to be withdrawn were given a s.c. dose of naloxone 10 mg kg^{-1}, two other groups were given a s.c. dose of saline 0·9% and the fifth group of animals were injected with an acute dose of morphine (10 mg kg^{-1} in saline). All animals were then infused for a further 60 min with the radioactive solution before being killed. During the infusion of radioactive marker all animals were partially restrained in Bolman cages.

Determination of intestinal transit

Intestinal transit was determined by measuring the progression of the non-absorbed radioactive marker through the small intestine. After the 90 min infusion was completed, animals were killed by exposure to cyclopropane gas (80–90%) in a closed chamber. Surgical anaesthesia was rapidly induced and death occurred within 30 s without causing any obvious signs of trauma to the internal organs (Paton & Payne 1968; Atkinson & Lee 1973). The abdominal cavity was then opened by a midline incision and the digestive tract ligated at the lower oesophageal sphincter, pylorus, ileo-caecal valve and the junction between the caecum and colon. The whole gastrointestinal tract from stomach to anus was removed from the animal and transferred to a longitudinal Perspex trough containing warm saline. Care was taken in handling the gastrointestinal tract to avoid stretching the tissue or displacement of luminal contents. The trough was pulled by an electric motor at a rate of 10 cm min^{-1} at a constant depth of 4·6 cm under a crystal scintillation detector (type DMI-2 Nuclear Enterprises), equipped with a 6 mm slit collimator and connected to a counter ratemeter (type MS 310E, J & P Engineering). The resultant radioactivity profile was displayed on a chart recorder also running at a speed of 10 cm min^{-1}. The remainder of the carcass was monitored using a scintillation meter (type 5·40 Mini-Instruments), to ensure all radiation used was contained within the excised gastrointestinal tract. Any faeces produced throughout the experiment was collected, monitored in a similar way to the intestine and analysed for any radioactivity.

Analysis of radioactivity profiles

The radiation profiles from the chart recorder were divided into 13 sections. These consisted of stomach, 10 equal sections of small intestine from the pylorus to the ileo-caecal valve, caecum and colon, including any faeces collected throughout the experiment. The area of each section of the profile was determined gravimetrically and expressed as a percentage of the total area of the radioactivity profile. Results were expressed as histograms of the percentage of radioactivity in each of the 13 sections for each group of rats.

Calculation of intestinal transit

Intestinal transit was quantified using the position of the leading edge of the infusion and the position of its geometric centre (G.C.). The G.C. method of analysing the gastrointestinal distribution of a radioactive marker has been previously described by Miller et al (1981). It is the weighted mean of the distribution along the intestine and is calculated by the equation $G.C. = \Sigma$ (fraction of ^{99}Tc per segment × segment number). The Mann Whitney U test was used to assess whether pairs of G.C.s were significantly different from each other.

Drugs and materials

Cyclopropane (B.O.C. Gases), morphine HCl (M & B), naloxone HCl (Sigma), pentobarbitone sodium (Sagatal, M & B), ^{99}technetium (Tc) sulphur colloid, liquid phase (Amersham International), Vitalife (Boots).

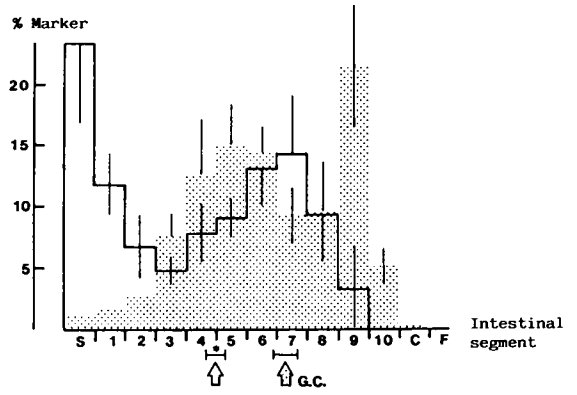

FIG. 1. The unshaded area shows the distribution of radioactive marker along the length of the intestine of non-dependent rats injected with a single dose of morphine (10 mg kg^{-1} s.c.). The shaded area is the distribution in the saline-injected control group. Lines associated with the % of marker in each segment indicate the s.e.m., n = 6 for both morphine and control groups. The Geometric Centre (G.C.) of the morphine-injected group is positioned significantly proximal to control (*$P < 0.01$). G.C. bars indicate the s.e.m.

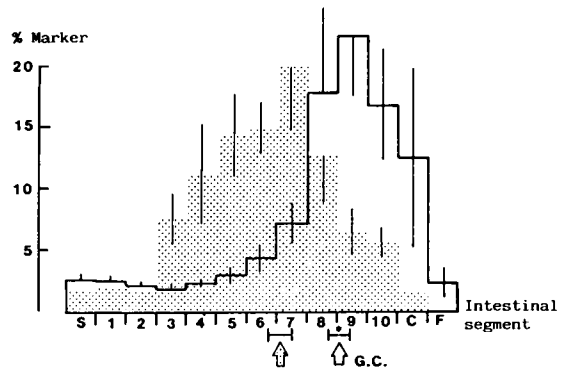

FIG. 3. The unshaded area shows the distribution of radioactive marker in withdrawn animals (morphine emulsion, naloxone s.c.) compared to non-dependent (emulsion only, naloxone s.c.). The position of the G.C. in animals undergoing morphine withdrawal is significantly distal to non-dependent ($P < 0.05$, n = 6 withdrawn n = 5 non-dependent groups).

Results

The distribution of the infusion in non-dependent animals injected with 10 mg kg^{-1} of morphine was markedly different to the distribution in saline-injected controls. Morphine caused a large reflux of infusion into the stomach. The levels declined along the upper small intestine but a second peak of marker was positioned in sections 6 and 7. The leading edge of the infusion reached section 9 compared with the caecum and colon in the non-dependent control group. The distribution pattern is summarised by the G.C. which was significantly proximal in the morphine-treated group compared with control ($P < 0.01$, Fig. 1).

The distribution of radioactive marker in morphine-dependent animals (morphine emulsion, saline) was similar to the distribution in non-dependent animals (emulsion only, saline). There was a gradual increase in radiation reaching a peak in section 7, then decreasing again towards the terminal ileum. The leading edge of the infusion reached similar positions in each group and the G.C.s were superimposable ($P > 0.05$, Fig. 2).

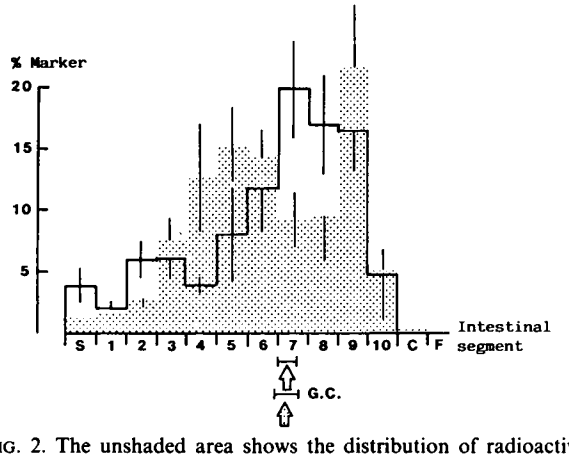

FIG. 2. The unshaded area shows the distribution of radioactive marker in morphine-dependent animals (morphine emulsion, saline s.c.) compared to non-dependent (emulsion only, saline s.c.) shown by the shaded area. There is no significant difference in the positions of the G.C.s ($P > 0.05$, n = 6 both groups).

Injection of non-dependent animals (emulsion only) with naloxone (10 mg kg^{-1}) did not change the position of the G.C. or the leading edge of the infusion compared to the non-dependent control group (emulsion only, saline $P > 0.05$). However, naloxone caused a marked change in distribution of the infusion in dependent animals compared with the non-dependent group (emulsion only, naloxone). There were fairly small amounts of radioactive marker in all intestinal sections until section 7 where amounts began to increase reaching a peak at section 9. This then gradually decreased throughout the remaining intestine. The faeces collected during the experiment contained some of the radioactive marker. The position of the G.C. was significantly distal to control ($P < 0.05$, Fig. 3).

During withdrawal the animals defaecated small, hard pellets for approximately 10 min after naloxone injection. This then changed to persistent diarrhoea. Other visible signs of withdrawal were body shakes, teeth chattering, urination, ejaculation and increased production of lacrimal fluid.

Discussion

The present results demonstrate clearly that tolerance occurs to the antitransit effect of morphine and that abrupt withdrawal from morphine induces a decrease in transit time.

In 1926, Miller & Plant stated that the intestine does not become tolerant to morphine. This was based on their finding that the morphine-induced rise in intraluminal pressure recorded from the ileum of conscious dogs remained undiminished throughout chronic morphine treatment. This, together with clinical observations has maintained the assumption that morphine does not induce tolerance to its constipating action (Jaffe 1985). However, there are two similar reports showing that the intestine of conscious rats becomes incompletely tolerant to the antitransit effect of morphine (Burks et al 1976; Weisbrodt et al 1977). Additionally there is indirect evidence based on myoelectric recordings indicating morphine induces tolerance in the rat as well as dog intestine (Kuperman et al 1987; Weisbrodt et al 1980). These studies demonstrated that the dose regimens employed induced only partial tolerance. The present results confirm

that tolerance does occur in the intestine and show additionally that it is both rapid and complete if the total dose is given as a slow release preparation rather than repeated doses. This is revealed by comparing the distribution profiles of the radioactively labelled infusion in animals treated with morphine for only two days with the profile in the non-dependent group. In both groups the leading edge of the infusion was in the same position and the G.C.s were superimposable. To be certain, however, that these results represent tolerance it was essential to establish that the method was able to detect the antitransit effect of a single acute dose of morphine. A variety of experimental approaches have been used previously to demonstrate this as a major constipating action of morphine (Galligan & Burks 1983; Stewart 1984; Gmerek et al 1986; Manara et al 1986) and indeed the present method demonstrates this well. In addition the presence of relatively large amounts of marker in the stomach and duodenum following acute administration of morphine is of interest and indicates that morphine stimulates retroperistalsis in the upper small intestine.

Morphine produces inhibition of transit by acting both centrally (Margolin 1963; Parolaro et al 1977; Stewart et al 1978; Schulz et al 1979; Galligan & Burks 1983) and locally in the intestine itself (Tavani et al 1980; Bianchi et al 1983; Manara et al 1986). Apart from affecting the muscle of the intestine via its intrinsic and extrinsic nerves, morphine also acts on the small intestinal mucosa to stimulate net fluid and electrolyte absorption (Lee & Coupar 1980; Warhurst et al 1984), an effect that further increases constipation. It is relevant that tolerance occurs to this proabsorptive effect in the rat within two days also (Coupar et al 1988; Warhurst et al 1984).

Adaptation to the effects of morphine on the intestine is also shown by the production of diarrhoea in tolerant animals upon withdrawal of morphine. This withdrawal sign is the opposite to the acute effect of morphine and its intensity is an indirect measure of the degree of physical dependence induced. It has been assumed from in-vitro experiments only that withdrawal from morphine stimulates intestinal motility. Although diarrhoea is a frequent symptom of opiate withdrawal there have been no studies to measure the change in transit and distribution of contents along the length of the intestine during withdrawal. In the present experiments morphine-withdrawal was induced abruptly by injecting tolerant animals with the opioid antagonist, naloxone. This procedure would displace endogenous opioids from their receptor sites, therefore non-dependent animals injected with naloxone were also included in the study for comparison. The results showed no difference in either the position of the leading edge of the meal or its G.C. This indicates that endogenous opioids do not have a dominant role in the control of intestinal transit.

The characteristic side-effects of morphine withdrawal were observed when tolerant animals were injected with naloxone (Collier et al 1972). Within 60 min, the leading edge of the infusion had travelled through the small bowel, caecum, colon and anus, whereas in control animals given naloxone it had just reached the caecum. The G.C.s indicated that the passage of marker in withdrawn animals was further when compared with control groups. The withdrawal response demonstrated a marked increase in transit possibly due to increased motility, but this could also be due to increased intestinal secretion.

It has been shown that several neurotransmitters are released from the guinea-pig ileum during withdrawal from morphine. There is very limited information available concerning the cause of morphine-withdrawal diarrhoea in the rat. Indirect evidence indicates that acetylcholine, 5-HT and prostaglandins are released since antagonists to these substances inhibit naloxone precipitated diarrhoea (Collier et al 1972; Francis et al 1978). All three substances influence intestinal motility but may further promote diarrhoea by stimulating intestinal fluid secretion. There is evidence that 5-HT and prostaglandins are released in the intestine following injection of naloxone to morphine-dependent rats. The endogenous release of these secretagogues is associated with blockade of fluid absorption from the jejunum and stimulation of fluid secretion into the lumen of the colon (Beubler et al 1984). Two independent studies confirm that the efficiency of the rat intestine to absorb fluid and NaCl decreases markedly during withdrawal from morphine (Chang et al 1984; Warhurst et al 1984).

The results of this study clearly demonstrate that the intestine does adapt to chronic exposure to morphine. This is manifest by a tolerance to and a dependence on the antitransit effect of morphine. Although the mechanisms of the morphine-induced changes have not been investigated, the clear-cut effects revealed by the presently described method provide the basis for further studies. This is important since therapeutic treatment aimed at inhibiting intestinal motility as well as preventing the loss of intestinal fluid should be of value in helping addicts to accept detoxification.

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