Morphine Tolerance and Dependence in the Rat Intestine

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ABSTRACT

There has been no previous demonstration of opioid tolerance and dependence with respect to the propulsive and contractile activities of the gut in vivo. In the experiments described herein, morphine was administered continuously (1 mg/kg/hr s.c., 72 hr) and/or by bolus injection (2 mg/kg) and intestinal motility and transit were evaluated in unanesthetized rats. Tolerance in intestinal motility (contractions) and propulsion (transit) was measured in two ways, i.e., by measuring the time required for motility and propulsion to return to control values and by measuring the loss of effectiveness of bolus morphine administered to animals receiving continuous infusion of the opiate. The dose of morphine chosen for continuous administration (1 mg/kg/hr s.c. via Alzet minipumps) was based on the dose at which morphine inhibited intestinal propulsion by 50%. Morphine (1 mg/kg/hr) decreased the frequency of contractions in, and propulsion along, the small bowel and colon and produced mild antinociception. The frequency of duodenal and colonic contractions returned to normal within 13 to 16 hr. After 24 hr of morphine treatment, the inhibitory effects of bolus doses of morphine on motility and transit were diminished; the effects were eventually lost (48 hr). Similarly, the antinociceptive effects of bolus doses of morphine were diminished by 18 hr and lost by 24 hr. Naloxone (0.1 mg/kg s.c.) given to morphine-tolerant animals (72 hr) resulted in an increase in the frequency and amplitude of contractions in the colon, an increase in the propulsive activity of the small intestine and colon and diarrhea. These results provide direct demonstration of opioid tolerance and dependence of contractile and propulsive activity in the rat intestine in vivo.

Repeated administration of morphine and related opiates results in tolerance and dependence. The presence of tolerance is demonstrated experimentally by progressive loss, over time, of opiate responses. Dependence is assessed by the generation of a characteristic withdrawal syndrome upon administration of a suitable antagonist, such as naloxone. Different pharmacological endpoints show differential development of both tolerance and dependence.

Gastrointestinal contractions (motility) and propulsion (transit) are influenced by morphine actions locally in the ENS of the gut, as well as by actions in the brain and spinal cord (Manara et al., 1976; Burleigh et al., 1981; Porreca et al., 1984). Low doses of systemically administered morphine in rats affect propulsion principally by peripheral actions in the ENS, whereas higher doses (those sufficient to induce significant antinociception) recruit CNS sites of action that augment the peripheral ENS effects.

The longstanding belief that tolerance does not develop to the constipating effects of morphine (Miller and Plant, 1926) is still widely accepted (Reisine and Pasternak, 1996), although several reports indicate that tolerance does develop to the antipropulsive effects of morphine in rat small intestine (Burks et al., 1976; Weisbrodt et al., 1977; Brown et al., 1988) and to the contractile effects of morphine in dog small intestine in vivo (Burks et al., 1974, 1976; Weisbrodt et al., 1980) and ex vivo (Burks and Grubb, 1974).

Most previous studies of opiate gastrointestinal effects have concentrated on the upper portions of the gastrointestinal tract, especially the small intestine. In intact animals, opiate actions in the colon are thought to be of great importance for opiate-induced delays in propulsion (constipation), but colonic actions of opiates have rarely been examined in animals or humans, because of technical limitations. There have been no published studies on the effects of prolonged opiate administration on the contractile or propulsive activity of the colon.

Our experimental strategy was to measure both contractions and propulsion in the small intestine and colon of the rat gastrointestinal tract during continuous administration of a dose of morphine with pronounced gastrointestinal effects but submaximal antinociceptive effects. The time course of development of tolerance was assessed by daily measure-
ments of contractions and propulsion. Additional bolus s.c. doses of morphine were administered to permit more precise measurement of changes over time during continuous morphine administration. Finally, naloxone was administered to evaluate the presence of dependence that could be attributed to chronic administration of morphine. We report herein that tolerance develops in the small intestine and colon with prolonged administration of morphine, when either contractile activity (motility) or propulsive activity (transit) is measured. Furthermore, naloxone-precipitated withdrawal contractions and withdrawal-induced increased propulsion were found to occur in both the small intestine and the colon in vivo, indicating that physical dependence also develops in both regions of the gastrointestinal tract.

**Materials and Methods**

**Animals.** Male rats (Sprague Dawley, 150–200 g; Harlan, Houston, TX) were used in all experiments. Animals were housed five per cage in a temperature-controlled room with a 12-hr light-dark cycle (lights on at 7:00 A.M.). Food and water were available ad libitum. The animals were anesthetized with Equithesin (a pentobarbital/chloral hydrate mixture; 2.5 ml/kg for males and 2.0 ml/kg for females), and Silastic catheters were surgically implanted in the proximal duodenum 1 cm from the pyloric sphincter and in the proximal colon 1 cm distal to the ceco-colonic flexure. For transit, the catheters were tunnelled s.c. to the scapular region of the back, coiled and placed in a 1-ml syringe body, cut 1-cm tall, with the flange secured under the skin with a purse-string suture. For motility, the catheters were tunnelled to the scapular region of the back and connected s.c. to a specifically fabricated nylon plug. Surgery was performed in a dedicated clean area, with sterile instruments. The animals were allowed to recover for 3 to 5 days before experiments were done. All experimental protocols were approved by The University of Texas Health Science Center at Houston Animal Welfare Committee.

**Drug administration.** Morphine was administered by constant infusion (1 mg/kg/hr s.c.) via an Alzet osmotic minipump (Alza Corp., Palo Alto, CA) or by bolus administration (2 mg/kg s.c.). The dose for continuous administration of morphine was chosen to establish a steady-state concentration of morphine in the blood approximately equal to the D_{50}, the effective dose of morphine that results in 50% inhibition of transit, based on the following equation:

\[
\text{Infusion rate} = \frac{D_{50}}{4 \cdot T_{1/2}} = \frac{2 \text{ mg/kg}}{4 \cdot 0.5 \text{ hr}} = 1 \text{ mg/kg/hr}
\]

The dose-response curve for morphine-induced inhibition of small intestinal and colonic transit in rats is shown in figure 5. The D_{50} was calculated from the linear portion of the dose-response curve (20–80% of maximum), using a linear regression analysis.

**Gastrointestinal motility.** Contractions of the proximal duodenum and proximal colon were directly monitored by intraluminal pressure measurements via the previously implanted indwelling catheters, in unanesthetized, freely moving animals. Both frequency (contractions per minute) and amplitude (increase in pressure, in millimeters of water) were measured. In some cases, a motility index (frequency times mean amplitude) was calculated. Occasional movement artifacts were easily identified as spikes that appeared simultaneously in both recorded channels, and they were eliminated from data analysis. The animals were not fasted. Recordings were begun at 9 A.M. and continued for no more than 6 hr. To determine the time at which tolerance developed to the motility effects of morphine, we compared the frequency of contractions in control recordings with the frequency of contractions after morphine infusion in seven groups of animals, 0 to 6 hr, 6 to 12 hr, 12 to 18 hr, 18 to 24 hr, 24 to 30 hr, 48 to 54 hr and 72 to 78 hr after morphine infusion. Each animal served as its own control, and recording of intestinal motility was limited to 6 hr. From this series of experiments, it was determined that tolerance developed between 10 and 16 hr after the start of morphine infusion, so subsequent experiments included those hours of recording, along with 24, 48 and 72 hr. The latency to development of tolerance was defined as the time required for the intestine to return to within 10% of the frequency of contractions recorded during the control period (without morphine). Latency to development of tolerance was also assessed as the time required to lose the response of the intestine to a bolus dose of morphine. For the latter assessment, each animal was recorded for 3 hr at the time intervals given above, morphine (2 mg/kg s.c.) was administered and motility recording was continued for another 3 hr. Naloxone (0.1 mg/kg s.c.) was administered after 72 hr of infusion.

Catheters were connected to a low-compliance, nitrogen-driven, pneumohydraulic pump (Arndorfer, Madison, WI) for low-volume (1–5 ml/hr total) perfusion of the lumen with distilled water. Perfusion pressure was monitored with a Statham P23 pressure transducer, and changes in pressure were recorded with a Beckman R511 oscillographic recorder.

**Gastrointestinal transit.** The propulsive activities of the small intestine and large intestine were assessed by the G.C. method (Miller et al., 1981). Intraluminal catheters were implanted in the duodenum 1 cm from the pyloric sphincter and in the colon 1 cm from the ceco-colonic flexure. A radioactive marker that is not absorbed from the lumen of the intestine was instilled, and its distribution along the length of the small intestine and colon was assessed by calculating a weighted average of the distribution of radioactivity along the length of the gut (G.C.). The average was weighted by multiplying the amount of radioactivity in each segment of intestine by its segment number. Thus, radioactivity propelled to the distal bowel bore more weight than that in the proximal bowel, where it was instilled. Higher values of G.C. indicate greater propulsion of luminal contents, from G.C. = 1 (no propulsion) to G.C. = 10 (maximum propulsion). Animals were fasted for 18 hr. A nonabsorbable radioactive marker (51Cr, in the form of sodium chromate, in saline) was instilled directly into the small and large intestine via surgically implanted catheters. After 35 min, the animals were lightly anesthetized with ether and sacrificed. The small intestine, cecum and colon were removed, and the feces were collected. The small intestine was divided into 10 equal segments and the colon into five equal segments plus the feces, which served as segment 6. Each segment was placed in a test tube and counted for gamma radiation (1 min; TM Analytic, Chicago, IL). From the cpm, the G.C. of transit was calculated as

\[
\text{G.C.} = \frac{\sum \text{ cpm per segment} \cdot \text{segment no.}}{\text{total cpm}}
\]

To keep the concept of G.C. consistent, the equation for the colon data included a factor of 1.66 to maintain a range of G.C. = 1 to 10. Percent change in small or large intestinal transit was calculated as

\[
\% \text{ Inhibition} = \frac{\text{test G.C.} - \text{control G.C.}}{1 - \text{control G.C.}} \cdot 100
\]

To assess tolerance to the effects of morphine on small and large intestinal transit, six groups of animals (n = 8–10/group) were used; they received control treatment (saline pumps plus saline bolus), acute morphine treatment (saline pumps plus morphine bolus, 10 mg/kg s.c.) or chronic morphine treatment (morphine pumps, 1 mg/kg/hr s.c., plus morphine bolus, 10 mg/kg s.c., at 24, 48 or 72 hr or 6 days after the morphine pumps were implanted). The bolus (morphine or saline) was administered 20 min before chromium was instilled into the intestine; the animals were sacrificed 55 min later, and the G.C. of transit was assessed.

To assess physical dependence in terms of the propulsive activity of the gut, four groups of animals (n = 6–8/group) were used. The animals received control treatment (saline pumps plus saline bolus), naloxone control treatment (saline pumps plus naloxone, 0.1 mg/kg
s.c.), chronic morphine treatment (morphine pumps, 1 mg/kg/hr, for 72 hr, plus saline) or chronic morphine plus naloxone treatment (morphine pumps, 1 mg/kg/hr, for 72 hr, plus naloxone, 0.1 mg/kg s.c.).

Antinociception and behavioral withdrawal. One group of animals (n = 4) was implanted with pumps delivering morphine (1 mg/kg/hr), and antinociception was evaluated using the hot plate test before and after the administration of a bolus of morphine (10 mg/kg s.c.) injected 2, 6, 12, 18, 24 and 48 hr after implantation of the pump. The hot plate was at 55°C, and the endpoint used was the latency to lick the hind paw or scrotum or to jump to escape the plate. A cutoff period of 60 sec was used to prevent injury to the animal. Percent antinociception was calculated from the following equation:

\[
\text{% Antinociception} = \left( \frac{\text{test latency} - \text{control latency}}{60 - \text{control latency}} \right) \times 100
\]

Because the measured endpoint was antinociception, a higher bolus dose of morphine was necessary than in the experiments in which gastrointestinal endpoints were measured. These animals were also evaluated for physical signs of withdrawal after 72 hr of chronic morphine administration (1 mg/kg/hr). Naloxone (0.1 mg/kg s.c.) was administered and the following behaviors were scored: number of wet dog shakes/10 min, number of vocalizations/10 min and total loss of body weight over 1 hr. The quantity of fecal matter expelled as a result of naloxone-precipitated withdrawal was quantified by measuring the change in weight of a preweighed absorbent pad under the animal, by measuring loss in body weight for each animal and by noting the appearance of the feces as watery diarrhea, soft unformed stools or formed fecal pellets.

Statistics. Results were subjected to one-way analysis of variance followed by Newman-Keuls tests for multiple comparisons, using statistical software for pharmacological measurements (Tallarida and Murray, 1987). Statistical significance is indicated at P < .05 and at P < .01.

Results

Motility

Acute morphine administration. Bolus administration of morphine (2 mg/kg s.c.) resulted in an inhibition of the frequency of contractions in the small intestine (42.7 ± 7.6% inhibition of frequency, n = 8). In the colon, a morphine bolus resulted in an inhibition of contractions (37.6 ± 12.6% inhibition, n = 4) in 50% of the animals and a stimulation of contractions (34.7 ± 12.1% stimulation, n = 4) in 50% of the animals. Representative examples of the effects of acute administration of morphine (2 mg/kg s.c.) on the contractile activity of the proximal duodenum and proximal colon in naive animals are shown in figure 1.

Chronic morphine administration. Continuous administration of morphine (1 mg/kg/hr s.c. via an Alzet osmotic minipump) decreased the frequency and amplitude of contractions in both the small intestine and colon. To determine the time at which tolerance developed to the motility effects of morphine, we compared the frequency of contractions in control recordings with the frequency of contractions at various times after initiation of morphine infusion. Morphine infusion resulted in a significant inhibition of contractility in both the small intestine and colon within 1 hr, and its effects persisted for 12 to 15 hr. Tolerance to continuous administration of morphine in the contractile activity of the intestine was assessed by two means, i.e., by measuring the time required for the small intestine and colon to return to normal frequency of contractions and by recording the loss of effectiveness of bolus administration of morphine (2 mg/kg s.c.) in the presence of continuous administration of the opiate. Figure 2A illustrates the time (mean ± S.E.M., n = 5) required for the duodenum and colon to display a frequency of contractions not significantly different from control. Both the duodenum and colon returned to normal contractility within 13 to 15 hr, suggesting that the contractile activity of the intestine was tolerant to the effects of continuous morphine administration (1 mg/kg/hr s.c.). In addition, tolerance to the effects of bolus administration of morphine on contractions of the small intestine and colon are shown in figure 2B. Compared with control (time 0), the effect of a morphine bolus (2 mg/kg s.c.) was diminished after 24 hr and essentially lost after 48 hr of continuous administration of morphine. Interestingly, morphine (2 mg/kg s.c.) administered 24 hr after continuous infusion of the opiate resulted in stimulation of colonic motility in 100% of the animals tested, compared with stimulation in 50% of the animals tested during the control period. For that reason, the stimulatory effects of morphine are illustrated in figure 2B.

Naloxone-precipitated withdrawal. Physical dependence was demonstrated in animals treated with naloxone (0.1 mg/kg s.c.) after 72 hr of chronic morphine administration (1 mg/kg/hr s.c.). Naloxone administration resulted in frequent high-amplitude contractions in the proximal colon and proximal duodenum. A representative motility recording of these withdrawal contractions is shown in figure 3. Contractions of the proximal duodenum are shown in figure 3, top trace, and those of the proximal colon are shown in figure 3, bottom trace. Figure 4 illustrates the mean increase in the frequency of contractions (fig. 4A) and the motility index of contractions (amplitude times frequency) (fig. 4B) in the duodenum and colon in response to naloxone. The withdrawal contractions induced in the colon by naloxone administration

![Fig. 1. Intraluminal pressure measured in the proximal duodenum (top trace) and proximal colon (middle and bottom traces) in three different animals, illustrating the effects of acute administration of morphine (2 mg/kg s.c.) on rat duodenal and colonic motility. In the proximal duodenum, morphine decreased the frequency of contractions and interrupted the migrating motor complex. In the proximal colon, morphine increased the frequency and amplitude of contractions in some animals (four of eight animals, middle trace) and decreased the frequency and amplitude of contractions in others (four of eight animals, bottom trace).](image-url)
are demonstrated by the significant increase in the frequency of contractions and the increase in the motility index.

**Propulsion**

**Acute morphine administration.** The inhibitory effects of increasing doses of morphine (1–10 mg/kg s.c.) on propulsion in rat small intestine and colon are illustrated in figure 5. The D_{50} was used as the dose for continuous administration of morphine. For morphine inhibition of small intestinal transit, D_{50} was 1.95 mg/kg (95% confidence interval, 1.34–2.83 mg/kg); for large intestinal transit, D_{50} was 2.96 mg/kg (95% confidence interval, 2.13–4.12 mg/kg).

**Chronic morphine administration.** The development of tolerance to the antipropulsive effects of morphine was demonstrated by the return of normal propulsive activity in animals receiving continuous morphine administration (1 mg/kg/hr s.c.) and by the loss of effectiveness of bolus morphine administration (10 mg/kg s.c.) to inhibit transit in the small intestine and colon. In animals treated continuously with morphine (1 mg/kg/hr), small intestinal and colonic transit was significantly inhibited after 6 hr of infusion (G.C. of small intestinal transit in morphine-treated rats (1 mg/kg/hr, 6 hr) = 3.15 ± 0.31, G.C. of saline-treated rats (saline, 1 μl/hr, 6 hr) = 4.98 ± 0.42, P < .05; G.C. of colonic transit in morphine-treated animals (1 mg/kg/hr, 6 hr) = 1.65 ± 0.15, G.C. of saline-treated animals = 2.5 ± 0.22, P < .05, n = 4–7 rats/group). However, by 24 hr, tolerance had developed in the small intestine and colon to the antipropulsive effects of morphine (small intestine: morphine G.C. = 4.9 ± 0.48, saline G.C. = 5.11 ± 0.27; colon: morphine G.C. = 2.8 ± 0.15, saline G.C. = 2.47 ± 0.21; n = 4–7 animals/group).

The ability of a high bolus dose of morphine to inhibit small intestinal and colonic propulsion was evaluated in groups of rats subjected to chronic administration of morphine (1 mg/kg/hr) for 24, 48 or 72 hr or 6 days. Figure 6 shows the G.C. of transit in the small intestine and colon in animals receiving saline treatment (control), bolus morphine treatment (10 mg/kg s.c.) or chronic morphine treatment (1 mg/kg/hr s.c. plus bolus morphine, 10 mg/kg s.c.). Small intestinal transit was significantly inhibited by the acute administration of a bolus dose of morphine (10 mg/kg s.c.) to naive animals. Tolerance to the antipropulsive effect of bolus morphine on the small intestine was apparent at 6 days, when the G.C. for small intestinal propulsion was not significantly different from control but was significantly different from that with bolus morphine administration. These results are illustrated in figure 6A. The development of tolerance to the antipropulsive effects of morphine on colonic transit are illustrated in figure 6B. After 24, 48 or 72 hr of continuous morphine treatment, bolus administration of morphine failed to significantly inhibit colonic propulsion, suggesting that tolerance may have developed. However, the G.C. values at these time points were not significantly different from those with bolus morphine administration in naive animals. At 6 days, bolus morphine administration produced no significant change from control, but the value for the G.C. was significantly different from that with bolus morphine administration in naive animals. It is difficult to assess, therefore, when tolerance is apparent. Clearly, animals are tolerant by 6 days.

**Naloxone-precipitated withdrawal.** Physical dependence was also apparent in the propulsive activity of the gastrointestinal tract. Figure 7 compares the G.C. of transit for small and large intestinal transit in morphine-dependent animals treated with saline and morphine-dependent animals treated with naloxone (0.1 mg/kg s.c.). The G.C. of propulsion in the small intestine and colon was significantly increased by naloxone in morphine-dependent animals.

![Fig. 3. Representative recording of intraluminal pressure in the proximal duodenum (top trace) and proximal colon (bottom trace) in an animal treated continuously for 72 hr with morphine (1 mg/kg/hr s.c.). Naloxone (0.1 mg/kg s.c.) resulted in a disruption of the migrating motor complex activity in the proximal duodenum and an increase in the frequency and amplitude of contractions in the proximal colon.](image-url)
oxone administration to morphine-treated animals was also associated with physical signs characteristic of precipitated withdrawal, such as diarrhea, wet dog shakes and vocalization. These indices of withdrawal were evaluated for 30 min and quantified. The mean wet weight of diarrhea produced was $7.7 \pm 0.8$ g. The mean loss in body weight was $9.0 \pm 1.5$ g. The animals were observed to have $30 \pm 1.6$ wet dog shakes and $8 \pm 1$ vocalizations. The distribution of radiochromium along the length of the gut is illustrated in figure 8 and clearly shows enhanced propulsion of contents, especially through the colon.

**Antinociception**

Finally, for comparison, we evaluated the development of analgesic tolerance to continuous morphine administration (1 mg/kg/hr), with or without bolus administration of the opiate (10 mg/kg s.c.), using the hot plate test in a group of rats. Acute administration of morphine at the D50 for inhibition of intestinal transit (2 mg/kg s.c.) did not produce reliably measurable antinociception. However, continuous administration of the opiate (1 mg/kg/hr s.c.) resulted in approximately 40% antinociception after 12 hr (fig. 9). The antinociceptive effect of chronic morphine was diminished over time and was completely lost by 24 hr. This tolerance to the antinociceptive effects of chronic morphine treatment was also seen in the loss of effectiveness of bolus administration of morphine (10 mg/kg s.c.) in animals treated chronically with morphine (1 mg/kg/hr) (fig. 9).

**Discussion**

A systematic evaluation of the development of intestinal tolerance and physical dependence with the continuous administration of morphine was needed for two reasons. First, no evaluation of the effects of chronic opiate administration
on colonic motility or propulsion had been performed in vivo. Previous reports that show the development of tolerance in the intestine have been limited to the upper gastrointestinal tract (Burks et al., 1974, 1976; Burks and Grubb, 1974; Weisbrodt et al., 1977, 1980; Kuperman et al., 1987), but the colon is thought to be of primary importance in the manifestation of clinical gastrointestinal side effects of opiates, namely, constipation. Second, the impression persists that tolerance to the effects of morphine in the gastrointestinal tract does not develop (Reisine and Pasternak, 1996) or that it develops more slowly than for other endpoints, such as antinociception and respiratory depression (Ling et al., 1989).

Intestinal motility and propulsion are inhibited by doses of morphine that are 3 to 10 times lower than those required to produce antinociception (Porreca et al., 1984). Way (1993) emphasized the importance of using appropriate doses of opiates to evaluate tolerance and dependence in his review on the subject. In cases in which the dose of morphine is too high, the degree of tolerance is underestimated. Ideally, the degree of tolerance and dependence should be measured when morphine is no longer producing an agonist effect but suffices to prevent withdrawal hypersensitivity.

Doses of morphine in the present study were selected on the basis of dose-response data collected with the specific endpoints used experimentally, taking into account pharmacokineti c factors to produce relevant circulating levels of morphine. With the dosage of 1 mg/kg/hr, morphine resulted in inhibition of small intestinal and colonic motility and propulsion and produced mild antinociception. We compared the development of tolerance and dependence in the small intestine and colon, using both propulsion (transit) and motility (contractions) as endpoints, with the antinociceptive effects produced by morphine.

Development of tolerance to antipropulsive effects of morphine appeared to be time- and dose-dependent. During continuous infusion of a fixed dose (1 mg/kg/hr) of morphine, tolerance to a superimposed bolus dose of 2 mg/kg required 2 to 3 days. Tolerance to a larger bolus dose of 10 mg/kg required 6 days, although some degree of tolerance was apparent after 24 hr, because the effect of bolus administration of morphine failed to significantly inhibit propulsion. This observation coincides with previous studies of small intestinal electromyographical effects in dogs that involved escalating dose levels of continuously infused morphine (Weisbrodt et al., 1980). Those studies demonstrated that, even after tolerance developed to a particular dose level, infusion of a larger dose resulted in renewed morphine action, to which tolerance eventually developed (which could be overcome by subsequent additional increases in dosage). Together, previous and present studies suggest that tolerance is not absolute, at least within a practical range of morphine doses that can be evaluated in living animals. However, intestinal tolerance appears to progress over time even at a fixed dosing rate.

The intestinal effects of morphine can be elicited by actions at both CNS and ENS sites (Manara et al., 1976; Burleigh et al., 1981). Low doses of morphine that produce little antinociception act primarily at ENS sites; higher doses that elicit full antinociception act also at CNS sites. Data obtained with development of tolerance to antinociception and to antipropulsive effects of morphine suggest that tolerance occurs...
somewhat more readily at CNS than at ENS sites. In the case of 10 mg/kg bolus doses superimposed on 1 mg/kg/hr continuous infusion of morphine, for example, tolerance to antinociceptive effects of morphine occurred 18 to 24 hr after initiation of infusion, whereas tolerance to antipropulsive effects required 3 to 6 days. However, it is possible that, even within the brain, tolerance could occur at different rates for different endpoints (Ling et al., 1989). If tolerance develops more slowly at peripheral than at central sites, this difference could explain the clinical observation that tolerance to the constipating effect of morphine develops slowly. However, we are not aware of any quantitative measurements that rigorously document the rate at which tolerance to the constipating effect of morphine occurs in humans.

An interesting but unexplained aspect of motility changes with the onset of tolerance in the colon involved the relative incidence of inhibitory and stimulatory responses. In naive rats, bolus doses of 2 mg/kg morphine produced stimulation of colonic contractions in 50% of the animals and inhibition of contractions in the other 50%. After 24 hr of morphine infusion (1 mg/kg/hr), only stimulatory effects of the bolus dose (2 mg/kg) were observed. Tolerance to the stimulatory effect developed over the subsequent 24 hr. The data indicate that, during morphine infusion, tolerance to the inhibitory effect developed before tolerance to its stimulatory effect. This observation parallels the pattern previously observed in dogs (Weisbrodt et al., 1980), with different rates for development of tolerance to stimulatory and inhibitory events.

The most striking observation in the present study was elucidation of dependence in the intestine in vivo. Other investigators showed that guinea pig ileum strips taken from morphine-tolerant/dependent animals developed contraction responses when exposed to naloxone and that the phenomenon of dependence and naloxone withdrawal contraction could be produced in ileal strips completely in vitro (Schulz et al., 1982; Revzani et al., 1983; Lux and Schultz, 1986). Naloxone-induced contractions were found in vivo in our experiments. Additionally, our technique permitted functional assessment of the withdrawal contractions, in terms of propulsive activity of the small intestine and colon. Naloxone-precipitated withdrawal, at a time when contractile activity was stimulated, produced enhanced propulsion in the small intestine and in the colon. The increased contractions and propulsive activity of the colon were associated with increased fecal output and appearance of watery stools characteristic of diarrhea. In accord with previous observations of naloxone-precipitated morphine withdrawal (Collier et al., 1972; Wei, 1981), the increased fecal output explained most of the immediate loss of body weight that followed administration of naloxone in dependent animals.

Constipation associated with opiate administration is thought to be due to both the antipropulsive and antisecretory effects of morphine. The development of tolerance to the antisecretory effects of morphine has been demonstrated in the rat small intestine in vivo and in the mucosa of the guinea pig ileum in vitro (Warhurst et al., 1984; Vinayek et al., 1985; Lux and Schultz, 1986; Margaritis et al., 1993). In addition, the opioid antagonist precipitated characteristic behavioral signs of withdrawal and physical dependence (Collier et al., 1972; Wei, 1981).

We conclude that chronic administration of morphine in rats results in tolerance to the effects of morphine on all of the major gastrointestinal functions affected by morphine, i.e., contractions, mucosal transport and propulsion. Also, continuous administration of morphine produces a condition of morphine dependence, especially in the colon, manifested as increases in contractions after precipitated withdrawal. Withdrawal was associated with increased propulsive activity in both the small intestine and the colon, explaining both the appearance of diarrhea and the rapid loss of body weight in tolerant/dependent animals.

**References**


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