PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 154, 587-590 (1977)

Tolerance to the Effect of Morphine on Intestinal Transit[1] (39724)

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Introduction. The repeated or "chronic" injection of a narcotic analgesic, exemplified by morphine, often results in the phenomena of tolerance and physical dependence (1-3). In regard to the gastrointestinal tract, however, the responses to repeated administration of morphine are not clear. Tolerance to the effects of morphine on intestinal contractions *in vitro* appears to develop. The isolated ileum of the guinea pig is inhibited by morphine and tolerance develops to this inhibition (4). Also, tolerance develops to morphine's stimulation of dog isolated small intestine (5). On the other hand, results on the development of tolerance to the intestinal actions of morphine *in vivo* are meager and conflicting. Miller and Plant monitored intraluminal pressures from the intestines of unanesthetized dogs and found that tolerance did not develop to the stimulatory effects of morphine (6). In contrast, Thor *et al.* monitored the myoelectric activity of the small bowel of the unanesthetized dog and found that tolerance did develop to the stimulant action of morphine (7).

Motility functions of the small intestine can be investigated by following the transit of a nonabsorbable marker. Alterations in transit are believed to be secondary to changes in the contractile patterns of the small intestinal musculature. This study was designed to: (i) assess quantitatively whether acute injections of morphine affect intestinal transit in unanesthetized rats, and (ii) determine whether prior treatment of the rats with repeated injections of morphine produces tolerance to the observed effects.

Materials and methods. Intraduodenal catheters were implanted in 32 Sprague-Dawley rats (Sprague-Dawley, Madison, Wis.) weighing 203 ± 4 g (mean ± SE). Details of implantation have been reported (8). Briefly, the animals were anesthetized with 20 mg of ketamine hydrochloride (Ketaject, Bristol Laboratories, Syracuse, N.Y.) administered intraperitoneally. A tygon catheter, 0.51-mm inside diameter and 1.51-mm outside diameter, was implanted into the duodenum through a small incision made at the anterior surface of the greater curvature of the stomach. The other end of the catheter was passed between the skin and musculature at the midline incision to exit through a small cutaneous puncture made in the midscapular region. The abdominal incision was closed in two layers. The exposed end of the catheter was sealed with collodion and taped to the back of the neck.

The animals were allowed to recover for 3 to 5 days (all animals were eating at this time and were gaining weight). They were then divided into three groups. Members of group 1 (naive-saline) were injected subcutaneously with saline and intestinal transit was determined the same day. Rats in group 2 (naive-morphine) were injected with saline three times daily for 7 days and intestinal transit was determined on the 7th day. Rats in group 3 (morphine treated-morphine) were injected with morphine sulfate, 20 mg/kg, three times daily (8:00 AM, 4:00 PM, and midnight) for 7 days and intestinal transit was determined on the 7th day. All

[1] This study was supported in part by USPH Grants DA 00801 and AI 15361. Dr. Castro is the recipient of NIH Research Career Development Award AI 00087. Dr. Weisbrodt is the recipient of USPHS Research Scientist Development Award DA-00022.

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ISSN 0037-9727

injections were given subcutaneously.

On the day that intestinal transit was determined, each rat was injected subcutaneously with either saline or morphine sulfate, 20 mg/kg. In the case of group 3 animals, the morphine injection came at either 8:00 AM or 4:00 PM so that the established pattern of injections was maintained. Determination of intestinal transit was then initiated either 30, 60, or 120 min after injection of either saline (group 1) or morphine (groups 2 and 3). Intestinal transit was determined using a modification of the technique described by Summers *et al.* (9). The tape was removed from the backs of the rats and the catheter was opened by cutting the tip previously sealed with collodion. The catheter was cleared with 0.05 ml of 0.9% saline, followed by subsequent instillation of 0.2 ml of saline which contained approximately 0.5 μCi of $Na_2{}^{51}CrO_4$ (hereafter referred to as ^{51}Cr). Intraduodenal instillation of ^{51}Cr solution was followed immediately by flushing with 0.05 ml of saline. The volume of the saline flush was greater than the void volume of the catheter and was volume sufficient to wash out consistently more than 99% of the previously injected isotope. All animals were killed by cervical dislocation 25 min after instillation of the ^{51}Cr. Two milliliters of blood were immediately obtained by heart puncture and then the abdomen was opened. Silk was used to tie the small intestine at the tip of the catheter and at the ileocecal junction. Other ties were made at the junction of the cecum and colon, and at the pylorus while withdrawing the catheter from the stomach. The GI tract was cut at the point of the ties and carefully removed by cutting away the mesentery.

Once removed, the intestine was divided by metal clips into 10 equal segments of approximately 10 cm each beginning at the tip of the catheter and proceeding distally. The intestine was cut at these clips. Each segment, as well as the cecum and the blood samples, were placed in vials. The distribution of ^{51}Cr in the blood and throughout the small bowel and cecum was assayed by monitoring the radioactivity in each vial with a Searle Model 1195 γ-counter. Data were expressed as percentage of distribution of radioactivity present or traversing a given segment of intestine during the 25-min test period. The amount of radioactivity recovered from the 10 intestinal segments was considered 100%.

Results. Neither placement of the intraduodenal catheter nor the daily injection of morphine sulfate interfered with the animals' ability to eat and gain weight. The naive rats gained 18.4 ± 6.9 g during 7 days of saline injections. The morphine-treated rats gained 24.8 ± 5.4 g during the same period. During the 1st day of morphine administration, the animals appeared to be in a catatonic state. After the first 2–3 days, however, their behavior appeared identical to that of the naive rats.

All blood samples were free of radioactivity, thus the ^{51}Cr was not absorbed from the gut. Measurement of gut transit demonstrated that in the naive rats treated with saline (group 1), chromium (25 min after instillation) was distributed along the proximal 90% of the gut length (Fig. 1). Acute administration of morphine to naive rats (group 2) caused a marked change in the

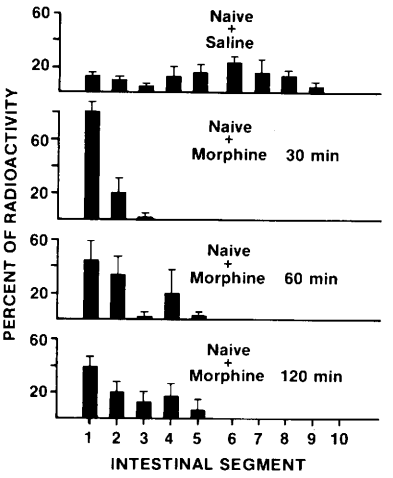

FIG. 1. Distribution of radioactivity along the length of the intestine 25 min after instillation of ^{51}Cr into the duodenum. Naive rats received saline for 7 days before testing. Time refers to the interval between injection of morphine and instillation of ^{51}Cr. Each segment corresponds to 0.1 of the total length of small intestine. Segment 1 contains part of the duodenum and segment 10 contains the terminal ileum. Means and standard errors are for 7, 4, 4, and 5 rats under the conditions of saline, and morphine at 30, 60, and 120 min, respectively.

distribution of ^{51}Cr in the GI tract. When ^{51}Cr was instilled 30 min after morphine administration, it never traversed more than 30% of the gut length. If the duration between morphine injection and ^{51}Cr instillation was 60 or 120 min, the chromium traversed 50% of the gut length.

Acute administration of morphine sulfate, 20 mg/kg, to the morphine-treated rats (group 3) also caused a decrease in transit of ^{51}Cr (Fig. 2). The decrease, however, was less than that produced in the rats comprising group 2. The ^{51}Cr front in rats tested 30, 60, and 120 min after the last injection of morphine traversed 70, 70, and 80% of the gut, respectively.

The means of the segments reached by the ^{51}Cr front are shown in Fig. 3. The front of the chromium traversed the shortest distance in the naive animals that were injected with morphine (group 2). The difference in levels reached was significant in the naive animals given saline (group 1) as compared to the naive rats given morphine (group 2) regardless of the interval of time (30–120 min) between morphine injection and testing. The distance traversed after morphine injection was also significantly less in the naive animals (group 2) compared to the morphine-treated animals (Group 3) at each respective time interval. There was no significant difference in the level reached in the morphine-treated rats that received morphine and were tested at 120 min compared to the naive rats that were injected with saline.

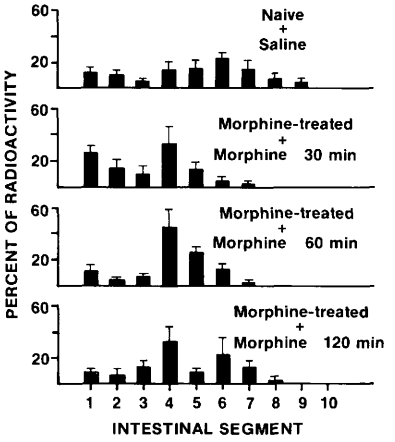

FIG. 2. Distribution of radioactivity along the length of the intestine 25 min after instillation of ^{51}Cr into the duodenum. Naive rats received saline and morphine-treated rats received morphine for 7 days before testing. Time refers to the interval between injection of morphine and instillation of ^{51}Cr. Each segment corresponds to 0.1 of the total length of small intestine. Segment 1 contains part of the duodenum and segment 10 contains the terminal ileum. Means and standard errors are for 7, 4, 4, and 4 rats under the conditions of saline, and morphine at 30, 60, and 120 min, respectively.

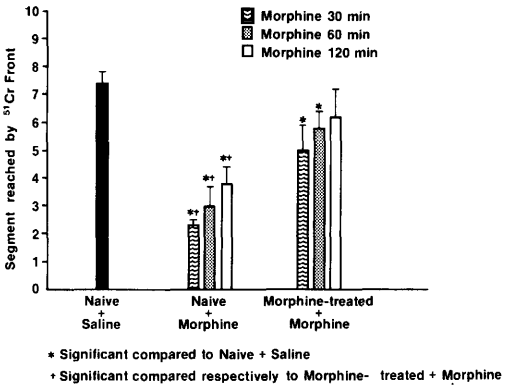

FIG. 3. The most distal segment reached by the ^{51}Cr 25 min after instillation into the duodenum. Naive rats received saline and morphine-treated rats received morphine for 7 days prior to testing. On the test day, either saline or morphine was injected 30, 60, or 120 min before instillation of the ^{51}Cr. A difference was considered significant if $P < 0.05$.

The mean of the most distal segments traversed by 50% of the chromium also showed differences among the three groups (Fig. 4). The distance was shortest in the naive rats that received morphine (group 2). The differences in distances between the naive rats given saline (group 1) and those given morphine (group 2) were significant at all three time intervals tested. At each respective time interval, the difference in distal traversed by 50% of the ^{51}Cr in the naive rats given morphine (group 2) was also significantly different from the distances traversed in the morphine-treated rats given morphine (group 3). There were no significant differences between the naive rats given saline (group 1) and the morphine-treated rats given morphine (group 3) regardless of the time interval.

Discussion. The techniques utilized in this investigation have several advantages. Two of the most important advantages are that

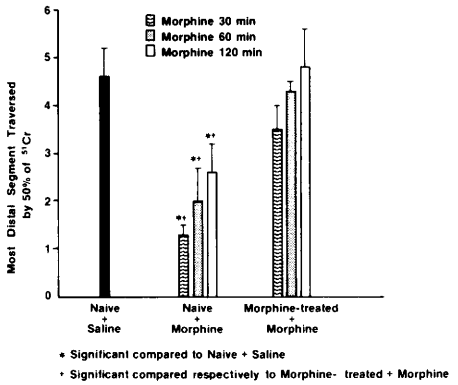

FIG. 4. The most distal segment traversed by 50% of the ^{51}Cr 25 min after its instillation into the duodenum. Naive rats received saline and morphine-treated rats received morphine for 7 days prior to testing. On the test day, either saline or morphine was injected 30, 60, or 120 min before instillation of the ^{51}Cr. A difference was considered significant if $P < 0.05$.

the animals are unanesthetized during determinations of intestinal transit and that the marker is administered directly into the small intestine, thus avoiding the variable of gastric emptying. Disadvantages of the technique are that we are following the movement of material for a relatively short time and that measurements of intestinal transit alone give little insight into the mechanisms by which transit may be altered.

Our results on transit in naive rats given saline and morphine compare well with those reported by others (8, 9). In addition, we found that the magnitude of the decrease in transit produced by morphine depended on the interval of time between the injection of morphine and testing. While transit was decreased when tested 120 min after morphine injection, the decrease was much less than when tested 30 min after morphine injection. The attenuative effects of morphine with time may be attributed to decreasing plasma concentrations since plasma levels of morphine are known to fall dramatically during the 2-hr period after injection (10).

The main observation we wish to stress is that, after repeated injections of morphine, the effects on intestinal transit normally produced by an acute dose of morphine were markedly diminished or lost. These data agree with the recent observations that chronic morphine treatment decreases the responses of dog intestine to acute doses of morphine *in situ* and *in vitro* (5). Our results differ, however, from those of Miller and Plant (6) and from the stated clinical impression that tolerance does not develop to the constipating effect of the narcotics (1, 2). In regard to constipation, our results must be viewed with caution since constipation could be caused by changes other than those related to transit through the small intestine. Nevertheless, our study represents an *in vivo* demonstration of morphine tolerance on transit through the small intestine.

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Received May 24, 1976. P.S.E.B.M. 1977, Vol. 154.