route of acetaminophen disposition in the rat, it does compete with nonmicrosomal sulfotransferase for the substrate. One report indirectly suggested that Fluosol hemodilution enhanced glucuronidation since the elimination half-life of phenytoin's primary metabolite, HPPH, was decreased [20]. But it has been suggested that phenytoin disposition may not be an appropriate marker of hepatic microsomal activity [21].

Thus it appears that hemodilution with either Fluosol or saline does reduce the acetaminophen sulfate Cl_M and V_d at 48 or 72 h, respectively. In addition, Fluosol uniquely alters the renal excretion of acetaminophen. With such limited information regarding the physiological responses to moderate hemodilution for several days, the mechanisms responsible for the effects are necessarily speculation. Only as more detailed investigations are reported will the speculation yield to understanding of the complex effects of hemodilution on drug disposition.

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Failure of (+)-naloxone to accelerate feline colonic transit

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Summary. To determine whether the colonic transit accelerating effect of (−)-naloxone (0.3 mg/kg, i.m.) is due to an action at opioid receptors or a direct pharmacologic effect, its enantiomer, (+)-naloxone (0.3 mg/kg, i.m.), was administered to cats and compared to saline control using colonic transit scintigraphy. Transit was not accelerated by (+)-naloxone. The effects of naloxone on colonic transit are thus stereospecific, and are probably mediated by opioid receptors.

Key words. (−)-Naloxone; (+)-naloxone; cat; transit; scintigraphy; colon.

The discovery of opioid immunoreactivity in the colon has led to the suggestion that colonic function is controlled, at least in part, by endogeneous opioids. Consistent with this theory, several studies have demonstrated altered colonic motility or accelerated transit after the administration of the narcotic antagonist (−)-naloxone to humans [1], cats [2, 3], and rats [4]. The improvement of chronic idiopathic constipation in 2 patients by (−)-naloxone further suggests that this antagonist may have clinical usefulness in certain colonic disorders [5]. However, since biological effects of (−)-naloxone not involving opioid receptors have been reported [6], it is necessary to demonstrate more conclusively that the accelerating effect of naloxone is an opioid receptor-mediated phenomenon. The (+)-enantiomer of naloxone has only $^1/_{1000}$ to $^1/_{10000}$ the opioid antagonist activity of (−)-naloxone [7], and has been used successfully to differentiate endogenous opioid receptor effects from the possible pharmacological actions of (−)-naloxone on its own [8]. In this study the effect of (+)-naloxone on colonic transit was compared to saline control. The dose, route of administration, and animal model were identical to a previous study from our laboratory which demonstrated accelerated transit with (−)-naloxone [2].

Materials and methods

Adult female cats, housed individually and under controlled temperature and lighting, were used for the study. Their diet was Purina cat chow and water ad libitum. Feline colonic transit scintigraphy was performed as previously described[9]. After induction of anesthesia with acepromazine (0.4 mg/kg, i.m.) and ketamine (4.0 mg/kg, i.m.), a 1.65-mm OD silicone catheter was surgically implanted into the cecum and tunnelled s.q. to the interscapular region, where it terminated in a luer stub adapter. Scintigraphy was not performed until 2 weeks after surgery, and no more than one study per week was done on any animal. On the day of the study, a fasted animal was lightly sedated with ketamine (50 mg, i.m.). 30 min after receiving an i.m. injection of the test agent, 25 μCi of ^{111}Indium diethylene triamine pentaacetic acid (^{111}In-DTPA) in 0.3 ml of 0.9% saline was instilled into the cecum. The test agents were (+)-naloxone (0.3 mg/kg) and 0.9% saline (0.5 ml). The dose of (+)-naloxone was the same as that which caused acceleration of transit with (−)-naloxone[2]. The distribution of the radionuclide was imaged for 6 h using a gamma camera interfaced to a minicomputer. The colonic images were divided into four regions of interest (ROI): 1 = cecum and ascending colon (CAC), 2 = transverse colon (TC), 3 = descending colon (DC), and 4 = excreted feces. After correction for background and decay, the counts in each region were quantitated. The activity in each ROI was expressed as a percent of the instilled radioactivity (time-activity curves). The area under the time-activity curves (AUC) was computed using the trapezoidal rule for the CAC, TC, and DC. A half-emptying time for the CAC was determined for each study. A geometric center was calculated for each aquisition, being the sum of the fraction of activity in each region multiplied by the number assigned to that region[2].

The sample size necessary to determine whether a significant difference was present or not was determined using methods outlined in Snedecor and Cochran[10]. The assumptions of inherent test variability and acceptable differences were based on prior research with this animal model and test methodology[9] (SD = 20.08%, delta = 25%, alpha = 0.05, and power = 0.80). Group values are expressed as the mean ± SEM. Student's t-test for paired data was used to determine whether significant differences were present. Results were considered significant if $p < 0.05$ (two-tailed test).

Results

There was no significant difference in the half-emptying times of the CAC after saline or (+)-naloxone

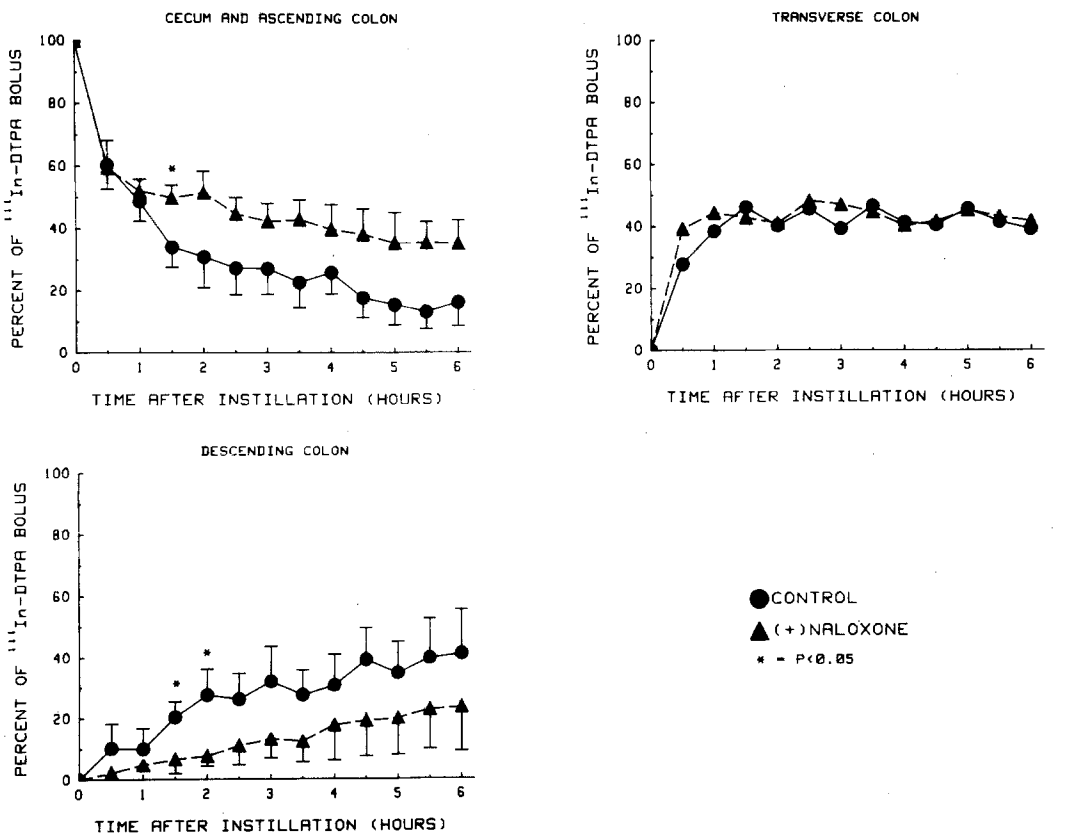

Figure 1. The effect of (+)-naloxone (0.3 mg/kg, i.m.) on colonic transit in the cat. (+)-Naloxone is compared to control (0.5 ml saline, i.m.) after instillation of 25 μCi of ^{111}In-DTPA into the cecum. Time activity curves for the cecum and ascending colon, transverse colon, and descending colon are shown in separate panels. Some error bars have been omitted for clarity.

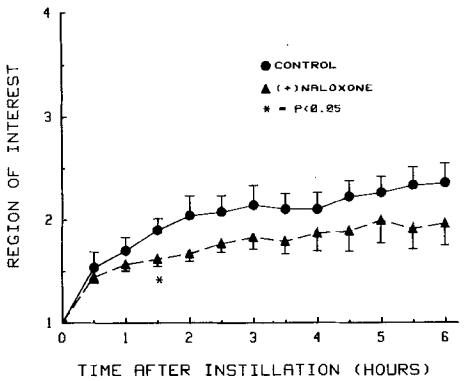

Figure 2. Effect of (+)-naloxone (0.3 mg/kg, i.m.) and saline (0.5 ml, i.m.) on feline colonic transit expressed as the progression of the geometric center against time after instillation of the ^{111}In-DTPA bolus. Some error bars have been omitted for clarity.

(1.42 ± 0.66 vs 2.41 ± 0.98 h, respectively, t = −0.848, $p > 0.05$). This is in contrast to (−)-naloxone, which accelerated CAC half-emptying from 1.14 ± 0.2 to 0.48 ± 0.2 h ($p < 0.05$) in the previous study[2]. There was also no difference in the AUC for (+)-naloxone (274 ± 35) vs control (187 ± 37) in the CAC ($p > 0.05$). There was no difference between the control and (+)-naloxone curves in the TC (fig. 1). Likewise, the AUC was similar for (+)-naloxone (253 ± 28) vs control (238 ± 35) ($p > 0.05$). In the DC, although delay occurred at 1.5 and 2 h after (+)-naloxone compared to control, there was no difference in AUC (74 ± 41 vs 161 ± 44, respectively; $p > 0.05$). The geometric center analysis (fig. 2) reveals little difference between control and (+)-naloxone, except for one isolated point at 1.5 h.

Discussion

The effects of naloxone on feline colonic transit are stereospecific. Transit is accelerated by (−)-naloxone[2], but not by (+)-naloxone.

If there is any effect of (+)-naloxone at all, it is a delay of transit. However, this delaying effect was certainly not impressive as only an occasional data point was significant on the time-activity curves. Measures such as half-emptying times and area under the curve, which are less susceptible to bias than individual t-tests, were not significantly different for (+)-naloxone and control.

In summary, because of the stereospecific effect of naloxone on colonic transit, and the absence of an effect of (+)-naloxone as compared to saline control, we conclude that the acceleration of transit by (−)-naloxone is at least in part an action at the opioid receptor, and not primarily a direct (non-opioid receptor) effect of the drug.

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