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Regional distribution of an opioid mechanism in the guinea-pig isolated intestine

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Opioid receptor blockade by naloxone enhanced peristalsis in isolated segments from the guinea-pig duodenum, jejunum and ileum, increasingly aborally. Thus, an increase in the influence of an intrinsic opioid mechanism may be responsible for the decrease in the level of peristaltic activity, observed aborally. However, the peristalsis of ileal segments which worked against their closed distal end was enhanced by naloxone to a similar degree as normal peristalsis.

Proximal parts of the guinea-pig small intestine develop peristaltic contractions in vitro more easily than distal parts (Trendelenburg 1917). One possible explanation for this could be regional differences in the function of inhibitory neuromodulators, for example endogenous opioids, intrinsic to the intestinal wall.

However, opioids have been found at their highest concentration in the duodenum (Smith et al 1976), whereas enhanced peristalsis after opioid receptor blockade by naloxone was found in isolated segments from the guinea-pig ileum, but not in segments from the duodenum or jejunum (Kromer & Pretzlaff 1979). The latter results were obtained from segments preincubated in Krebs-solution containing naloxone and a comparison with drug-free control segments made. We now report on the response after application of naloxone to the organ bath and present evidence of influence of an opioid mechanism in the duodenum, jejunum and rectum.

Since it has been reported that naloxone reversed the 'fatigue' of continuing peristalsis but did not affect 'normal' peristalsis (Van Nueten et al 1976), we have tested the influence of naloxone on peristaltic activity that was significantly reduced by working against the closed distal end of the segment. In addition, we investigated whether there was any difference in the influence of naloxone upon application to the serosal compared with the mucosal surface of the segment.

MATERIALS AND METHODS

Adult male and female guinea-pigs were decapitated and the proximal 9 cm of duodenum, a 9 cm segment beginning at a point 15 cm distal from the ligamentum duodenocolicum (jejunum), the distal 9 cm of the ileum and a 9 cm segment beginning about 12 cm above the anus (rectum) were taken.

The lumina of the segments were flushed with 10 to 20 ml of a modified Krebs-solution (mM: NaCl 118; KCl 4·75; $CaCl_2$ 2·54; KH_2PO_4 1·19; $NaHCO_3$ 25; glucose 11; choline-HCl 0·02; bubbled with 95% O_2 and 5% CO_2). The open distal end of each segment was secured over a tube connected with a reservoir containing Krebs-solution, and the segment mounted in an organ bath at 37 °C (Trendelenburg 1917; for details see Kromer & Pretzlaff 1979). The reservoir was connected to a volumometer for recording of luminal volume displacement during peristalsis. The proximal end of the segment was closed with a thread which was tied to an isometric transducer for recording longitudinal contractions.

In other experiments, two adjacent ileal segments from each animal were compared. One segment was prepared and mounted as described above, the other was mounted with the closed distal end up. Only the longitudinal tension was measured, since aboral luminal volume displacement was prevented in the segments with the closed distal end which swelled like a balloon upon each peristaltic wave.

Over an equilibration period of 45 min, the baths were perfused with Krebs solution at 0·4 ml min^{-1}. Thereafter, the intraluminal pressure was increased and kept constant. The intraluminal pressures used are indicated in results.

After 10–20 min, when a steady pattern of peristalsis had been developed either (−)-naloxone or (+)-naloxone was applied to the serosal or mucosal surface. Application to the mucosal surface (lumen) at the start of the filling phase was through a small

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tube, the tip of which reached the open distal end of the segment via the tube connecting the segment to the reservoir. The amount of substance necessary to achieve the desired final concentration was calculated allowing for the luminal volume of the segment and the volume of the lumen of the adjacent tube, which may cause some dilution of the substance applied. Therefore, the final concentrations given in Fig. 2 for mucosal application may vary by a factor of up to 1·5.

In the evaluation of results, the times before and after drug application were subdivided into 15 min intervals, the number of peristaltic waves within each being related to the value obtained in the interval immediately preceding drug application (100% value). The significance of differences between the respective values obtained in segments from different gut regions were tested by Student's *t*-test.

The time course of peristaltic activity in control segments receiving (+)-naloxone was the same for all gut regions tested. Therefore, the data from controls were pooled.

RESULTS

(−)-Naloxone at 2×10^{-7} M, applied to the serosal surface of the segment, enhanced peristalsis in all regions tested (Fig. 1). Similar data were obtained with naltrexone (not shown). The influence of naloxone was greatest in the ileum, and least in the duodenum and rectum. However, the difference from controls ((+)-naloxone 2×10^{-7} M) was statistically significant for all regions (for *P*-values see legend to Fig. 1). Therefore, the influence of naloxone on peristalsis was stereospecific.

In the small intestine, the percentage enhancement of peristalsis by naloxone (see Fig. 1) increased from proximal to distal ends as the magnitude of basal peristaltic activity decreased. The basal peristaltic activity was: duodenum 64 s.d. 29%, jejunum 40 s.d. 14%, ileum 34 s.d. 16% and rectum 12 s.d. 4%. The difference between duodenal and ileal segments was significant ($P < 0{\cdot}05$). The coefficient of linear correlation (r) for the +15-min values and their respective basal activities was −0·71 for the ileum (n = 12), −0·51 for the jejunum (n = 14), −0·50 for the duodenum (n = 12) and −0·60 for the pooled data.

The degree of enhancement of peristalsis by naloxone declined with time but the difference between ileal and duodenal values remained statistically significant 90 min after drug application.

After mucosal application of naloxone to ileal segments with an intraluminal pressure of 2 cm water, 10 to 100 times more naloxone was needed to obtain an enhancement similar to that after serosal application (Fig. 2).

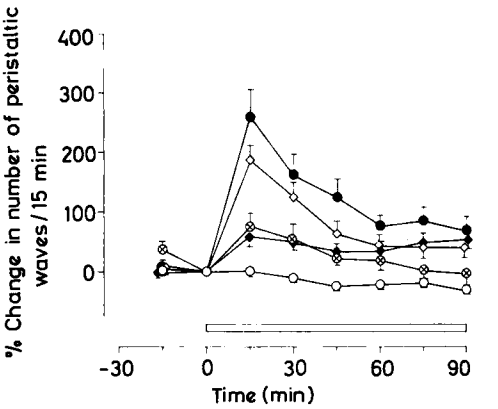

FIG. 1. Influence of naloxone (2×10^{-7} M) on peristaltic activity in isolated segments from the guinea-pig intestine. Experiments were performed at a sustained elevated intraluminal pressure equivalent to 3 cm water (duodenum, jejunum, ileum) or 10 cm water (rectum). Each value refers to the preceding 15 min interval. All values are expressed as a percent change (mean ± s.e.), compared with the 15 min interval immediately preceding naloxone application (set at 0 % change). The bar indicates the presence of (−)-naloxone (ileum ●; jejunum ◇; duodenum ⊗; rectum ◆) or (+)-naloxone (pooled controls; ○); n = 12–14.

P-values. All comparisons between values obtained within 15 min of (−)-naloxone application and the respective control value ((+)-naloxone) yielded *P*-values of <0·001. Differences between values obtained within 15 min of (−)-naloxone application to ileal and jejunal segments were not statistically significant. However, both groups differed from duodenal and rectal segments ($P < 0{\cdot}002$), the difference between ileal and duodenal segments being significant even 90 min after naloxone application ($P < 0{\cdot}02$).

A short-lasting spasm-like contraction after (−)-naloxone application was sometimes superimposed upon peristaltic waves in the isolated rectum. In such cases, the +15 min value was estimated.

In ileal segments that worked against their closed distal end and 2 cm water, basal peristaltic activity was decreased to 12 s.d. 8 peristaltic waves per 15 min, compared with 23 s.d. 7 peristaltic waves per 15 min, when the distal end of the segment was open (n = 9; $P < 0{\cdot}01$). But the percent enhancement by naloxone was the same in both groups (distal end open: 153 ± 26%; distal end closed: 195 ± 31%; mean ± s.e.; not significant). An example is shown in Fig. 3.

DISCUSSION

Enhancement of peristalsis by (−)-naloxone, but not (+)-naloxone, suggests the existence of an inhibitory opioid mechanism within the intestinal wall. The finding that naloxone significantly enhances peristal-

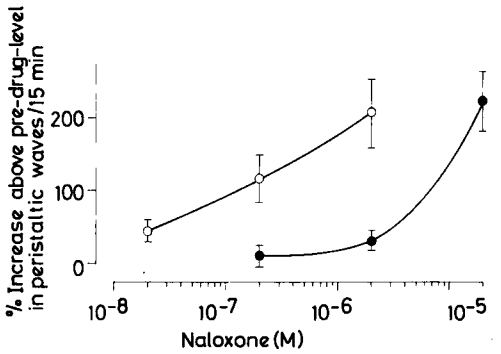

FIG. 2. Influence of (−)-naloxone on peristaltic activity in isolated ileal segments after serosal (○) or mucosal (●) application. Experiments were performed at a sustained elevated intraluminal pressure equivalent to 2 cm water. Ordinate: Percent change above pre-drug-level in peristaltic waves per 15 min (mean ± s.e.) n = 9–19.

sis in the ileum compared with the duodenum is perhaps in conflict with the earlier findings of Smith et al (1976), who found the highest concentration of enkephalins in the duodenum. However, a high content may correspond to a lower functional significance of that pool. Basal peristaltic activity was highest in the duodenum, which was least affected by naloxone, and vice versa in the ileum. Thus, an increasing degree of naloxone influence may possibly correspond to an increasing degree of basal activation of an inhibitory opioid mechanism aborally. A

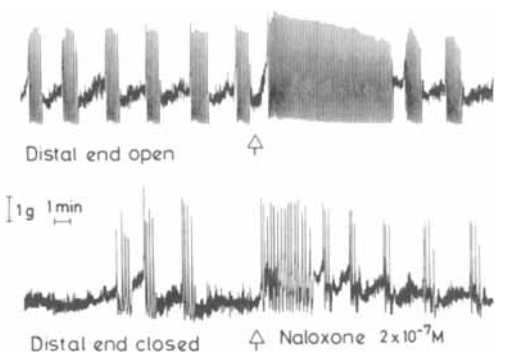

FIG. 3. An example of the naloxone influence at 2×10^{-7} M upon peristalsis during a sustained elevated intraluminal pressure (2 cm water). Longitudinal tension of two adjacent ileal segments from one animal. Each record shows both a 15 min period preceding and after naloxone application (△). Upper trace: control segment (distal end open). Lower trace: a segment in which expulsion of the luminal Krebs-solution was prevented (distal end closed).

greater number or sensitivity of opioid receptors in more aboral parts could also be responsible (see Schulz 1978). However, it is difficult to compare the influence of naloxone in the isolated rectum with its influence in segments from the small intestine, since different intraluminal pressures were needed to induce peristalsis.

To our knowledge, opioids are the only inhibitory substances intrinsic to the intestinal wall so far shown to participate in the control of peristalsis in vitro. Noradrenergic nerves enter the intestinal wall from the sympathetic plexus (see Kosterlitz & Watt 1975; Costa & Furness 1979) whilst a functional significance of purinergic neurons (see Burnstock 1972) in the control of peristalsis in vitro has not as yet been demonstrated.

Ileal segments working against their closed distal end displayed less peristaltic activity compared with segments which were allowed to expel their luminal content. If intestinal opioids were responsible for this state of fatigue, naloxone should have enhanced peristalsis more in the distally closed segments, however, the percentage enhancement of peristalsis was the same in both groups. Thus, naloxone appears to affect normal peristalsis rather than counteract fatigue.

When naloxone was applied to the mucosal surface, a much higher concentration was necessary to achieve a degree of enhancement of peristalsis similar to that after serosal application. This is consistent with our unpublished data, showing that [^{3}H] naloxone penetrates very slowly across the intestinal wall in vitro. Even after an incubation time of 120 min at 21 °C, the luminal ^{3}H concentration was only 1/10 of that applied serosally (2×10^{-9} M [^{3}H] naloxone). On the other hand, naloxone's action has an immediate onset after serosal application. Since naloxone is lipophilic (Kaufman et al 1975) and is rapidly absorbed in vivo (see Berkowitz 1976), one possible explanation for the lower influence of naloxone after mucosal application might be that some accumulates within the mucosa.

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