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Morphine-Like Action of Enkephalin Analog FK 33-824 on Motility of the Isolated Rat Colon

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ABSTRACT

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Actions of the synthetic met-enkaphalin analog FK 33-824 were compared to normorphine on intraluminal pressure of the isolated rat colon. Phasic and tonic contractions of isolated colonic segments in Ringer-Tyrode's solution under standard conditions were measured by intraluminal perfusion manometry. FK 33-824 or normorphine induced substantial tonic contractions of colonic muscles which lasted about 6 min. The maximum tonic contraction occurred at 10^{-5} M normorphine or 10^{-6} M FK 33-824. Naloxone (10^{-6} M) and Mr 2266 alone or in combination inhibited competitively the tonic contraction induced by FK 33-824 with a similar potency ratio, whereas 10^{-6} M norepinephrine or 10^{-8} M isoproterenol inhibited it noncompetitively. Tetrodotoxin (10^{-6} M) counteracted tonic contractions due to FK 33-824 or normorphine in all parts of the undissected colon but only in the proximal segment of the dissected colon and enhanced it in the middle and distal colonic segment. Neither 10^{-5} M atropine sulfate nor 10^{-6} M methysergide interfered with the action of FK 33-824 or normorphine. FK 33-824 produced morphine-like motor actions in the isolated rat colon evidently by stimulation of opiate receptors, suggested to be located in nervous and muscular tissue in the proximal and solely in muscular tissue in the mid and distal colonic segment. The neural pathway stimulated by FK 33-824 or normorphine is not identified. The data suggest it to be noncholinergic, nonadrenergic and nonserotoninergic.

The biological actions of enkephalins on gastric and small bowel motility of different species have been shown to have similarities to those of opiates (Hutchinson *et al.*, 1975; North, 1976; Sprügel *et al.*, 1977; Konturek *et al.*, 1978; Thor *et al.*, 1978; Schulz *et al.*, 1979). Opiate actions on colonic motility in man and animals have been widely studied (Adler *et al.*, 1942; Painter and Truelove, 1964; Garrett *et al.*, 1967; Rinaldo *et al.*, 1971). Little information is available on enkephalin actions (Stacher *et al.*, 1979). In human and canine colon (Adler *et al.*, 1942; Rinaldo *et al.*, 1971) and in isolated colonic muscle strips of rats (Gillan and Pollock, 1976), opiates immediately increase the basal intraluminal tonic pressure and the amplitude of phasic contractions. When tonic pressure returns to basal levels, a series of high-pressure contractions with decreasing amplitudes occur at intervals of approximately 1 min. It was suggested that enkephalins might exert opiate-like actions on colonic muscles (Stacher *et al.*, 1979).

The purpose of this study was to compare the effect of the synthetic met-enkephalin analog FK 33-824 to that of normorphine on the motility of the isolated rat colon and to study the mechanism of action.

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Materials and Methods

Animal preparations. Adult male Wistar rats (320 ± 30 g) were used. Under ether anesthesia, the colon was removed (without the rectum) and its lumen was cleaned with warm Ringer-Tyrode's solution. The colon was then trimmed to three equal sized pieces which represented proximal, mid and distal colon. The orad end of each piece was labeled with a colored silk thread, then placed in Ringer-Tyrode's solution (composition: 137 mM NaCl; 5.4 mM KCl; 0.5 mM $MgCl_2$; 1.8 mM $CaCl_2$; 11.9 mM $NaHCO_3$; 0.4 mM $NaH_2PO_4 \cdot 2\ H_2O$; and 5 mM glucose). The organ bath fluid was bubbled with 5% CO_2 95% O_2 and was maintained at a pH of 7.4 and a temperature of 37°C. The bath level was kept constant at 40 ml by a suction pump, the bath continuously renewed by a roller pump-driven flushing system.

Intraluminal pressure measurement. A syringe pump-driven perfusion manometric system with intraluminally located pressure sensor devices was used to assess phasic and tonic pressure changes in the three colonic segments of each rat. The intraluminal pressure sensor device is shown in figure 1. It consists of a constantly perfused (5 ml hr^{-1}) polyethylene catheter (outside diameter, 1.2 mm; inside diameter, 0.8 mm) with an open tip. The distal 3 cm of these catheters were cast in a dental acrylic tube (outside diameter, 2.5 mm) in order to reduce the compliance of the system. The catheter eye placed on the surface of a spindle shaped thickening (outside diameter, 5 mm) at the distal end of the acrylic tube was used to increase the sensitivity of the pressure measuring system by bringing the sensor part close to the colonic wall. The sensor catheters were perfused with distilled water by

ABBREVIATIONS: TTX, tetrodotoxin; AUC, area under base-line pressure-time-curve.

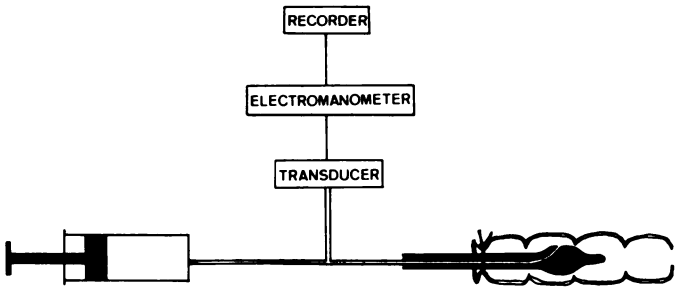

Fig. 1. Intraluminal pressure measuring system.

a syringe pump (Unita I, Braun Melsungen) and had an end to side connection with the volume displacement transducer (Statham P 23b). All pressure changes were measured with Philips electromanometers (XM 5101) and recorded by a Cardiopan 8R multichannel writer (Liechti Ltd., Berne, Switzerland).

Rhythmic intraluminal pressure changes are called phasic contractions, a base line pressure increase of more than 1-min duration with or without superimposed phasic contractions is called tonic contraction.

Design of studies. The isolated colonic pieces were kept in the organ bath for 15 min in order to establish a steady state in tonic and phasic intraluminal pressure activities. Thereafter, the different test substances were added to the organ bath in a volume not exceeding 0.2 ml or given as a long term perfusion with the substances added to the Ringer-Tyrode's solution. The washing out of the drugs occurred by replacing the organ bath with fresh Ringer-Tyrode's solution within 2 min and a subsequent rinse of the organ preparation with fresh nutrient at a rate of 20 ml per min for at least 20 min.

The following substances were used: FK 33-824 (Tyr-D-Ala-Gly-MePhe-Met(O)-01), a synthetic met-enkephalin analog (Sandoz Ltd., Basle, Switzerland); naloxone (Winthrop Laboratories, Inc., New York); normorphine, Mr 2266 [(−)2-(3 furymethyl)-5,9-diethyl-2'-hydroxy-6,7-benzomorphan] (Böhringer Ingelheim Ltd., Elmsford, NY); TTX (Serva, Heidelberg, W. Germany); norepinephrine tartrate (Serva); isoproterenol (Serva); propranolol (Serva); phentolamine (Ciba Pharmaceuticals, Basle, Switzerland); atropine sulfate (Ph.H); acetylcholine chloride (Baeschlin, Winterthur, Switzerland); methysergide (Sandoz, Ltd., Basle, Switzerland); and serotonin-HCl (Sigma Chemical Company, St. Louis, MO).

In dose-response examinations, several doses were administered in a random sequence in the same organ preparation. The dose-response relationship of test drugs on tonic colonic muscular contraction was assessed by calculating the average area under the base-line pressure increase during 5 min for each dose level. The results are given in arbitrary units.

Actions of FK 33-824 and normorphine and the interaction of opiate receptor antagonists on intraluminal colonic tone and phasic activities. Dose-response examinations were performed with either FK 33-824 (10^{-8}–10^{-5} M) with and without 10^{-6} M naloxone or Mr 2266 or with normorphine (10^{-8}–10^{-4} M) with and without 10^{-7} M naloxone or 10^{-8} M Mr 2266.

The colonic preparations were first exposed for 5 min to the opiate receptor antagonist alone, then FK 33-824 or normorphine were added for a further 5 min. After a wash out period of 20 min, the stimulants were given alone, again for 5 min.

In a second series of tests, a dose-response examination with FK 33-824 (10^{-8}–10^{-5} M) without and with a combination of 10^{-6} M naloxone and 10^{-6} M Mr 2266 were performed in a similar manner.

Interaction of TTX with FK 33-824 or normorphine on intraluminal colonic tone. A 5-min stimulation with FK 33-824 (10^{-7}–10^{-6} M) or normorphine (10^{-7}–10^{-6} M) was performed after a 20-min pretreatment of colonic preparations with 10^{-6} M TTX. After a wash out period of 30 min, the respective stimulants were given alone for 5 min. The effects were calculated from the last 5 min of the pretreatment period with TTX and from the 5 min stimulation period in the presence and absence of TTX.

Interaction of atropine sulfate with FK 33-824, normorphine and acetylcholine on colonic intraluminal tone. The colonic segments were first stimulated with 10^{-5} M acetylcholine, 10^{-7} M FK 33-824 or 10^{-6} M normorphine for 5 min. After a 10-min wash out period, the preparations were exposed to 10^{-5} M atropine sulfate for 20 min. After a 10-min exposure, 10^{-5} M acetylcholine was added for 5 min. the preparations were then rinsed with an atropine-containing bath fluid for 5 min. Thereafter, the stimulation with 10^{-7} M FK 33-824 or 10^{-6} M normorphine was performed for 5 min in the presence of atropine. After a further wash out period of 30 min with ringer-Tyrode's solution, the preparations were stimulated with acetylcholine, FK 33-824 or normorphine for 5 min.

Interaction of norepinephrine, isoproterenol, phentolamine and propranolol with FK 33-824 or normorphine. Dose-response relationship was studied with FK 33-824 (10^{-8}–10^{-5} M) or normorphine (10^{-8}–10^{-5} M) with and without 10^{-7} and 10^{-6} M norepinephrine or 10^{-8} to 10^{-7} M isoproterenol. After a 5-min pretreatment with norepinephrine or isoproterenol, the different doses of the respective stimulants were added for 5 min and the preparation was then washed out for 20 min. After this procedure, the stimulants were given alone for 5 min.

For phentolamine or propranolol (both 10^{-6} M), a pretreatment period of 15 min was used. The subsequent stimulations occurred with 10^{-6} M FK 33-824 or normorphine with and without 10^{-7} M isoproterenol or 10^{-6} M norepinephrine.

Interaction of methysergide with FK 33-824, normorphine or serotonin. Dose-response relationship was studied with FK 33-824 or normorphine (both 10^{-8}–10^{-5} M), with and without a 30-min pretreatment with 10^{-6} M methysergide. The pressure changes were calculated from the last 5 min of the pretreatment period with methysergide and during the 5-min stimulation with FK 33-824 or normorphine, with and without methysergide.

As a control, the effect of 10^{-6} M methysergide on serotonin (10^{-8}–10^{-6} M)-stimulated colonic segments was observed using the same timing.

Statistical evaluation. The data were compared by the paired Student's t test, one-way analysis of variance, the symmetrical 3×3 dose parallel line assay, the symmetrical 2×2 dose parallel line assay and linear regression analysis (Colquhoun, 1971; Sachs, 1972).

Results

The motility pattern before, during and after exposure of the proximal colonic segment to 10^{-7} M FK 33-824, 10^{-6} M normorphine or 0.15 M saline is shown in figure 2. Normorphine as well as FK 33-824 induced a rapid tonic contraction with a subsequent rise in the strength of phasic contractions without a change in their frequency. Tonic and phasic reactions to both stimuli returned to basal levels within 6 min. Then high-pressure contractions occurred with a frequency of 1 to 2 min^{-1}. Similar reactions were observed in the mid and distal colonic segment. Saline did not alter the intraluminal basal pressure pattern.

Figure 3 shows the dose-response relationship of normorphine with and without naloxone or Mr 2266 on muscular tone of the proximal colonic segment in comparison to that of FK 33-824 with and without naloxone or Mr 2266. The maximum normorphine-stimulated tonic contraction occurred with a dose of 10^{-5} M. The comparative value for FK 33-824 was 10^{-6} M.

Naloxone or Mr 2266 givenwith FK 33-824 or normorphine shifted the dose-response curves of the agonists symmetrically to the right, indicating competitive antagonism. Such behavior was observed in the three colonic segments of each animal (n = 6) tested, The potency ratio obtained with FK 33-824 and Mr 2266 was 0.102 and was similar to that of FK 33-824 and naloxone with 0.108. The respective values for normorphine were 0.110 and 0.102, but the antagonist doses differed.

Figure 4 shows the dose-response relationship of FK 33-824

without and with 10^{-6} M naloxone + 10^{-6} M Mr 2266. The combination of both inhibitors caused a symmetrical shift of the dose-response curve to the right with a potency ratio of 0.115. The statistical evaluation of these results by the symmetrical 3 × 3 dose parallel line assay demonstrated that the average slope of the respective dose-response curves was significantly different from 0 (all P values obtained by *F* method: <.001), there was no significant deviation from parallelism (all *F* values less than 0.7) and no difference in curvature ($F < .9$), but a significant difference between the groups treated with and without opiate receptor antagonists ($P < .01$). No significant difference, however, was found between the extent of inhibition caused by the single opiate receptor antagonist and the combination of both opiate receptor antagonists ($P > .2$).

In the intact isolated colon, 10^{-6} M TTX reduced the stimulatory action of 10^{-6} M FK 33-824 in the proximal part from 44.7 ± 5.4 AUC to 13.8 ± 4.1 ($P < .005$), in the midcolon from 43.2 ± 4.6 to 11.83 ± 2.2 ($P < .005$) and in the distal colon from 17.5 ± 3.9 to 18.7 ± 2.5 ($P < .01$).

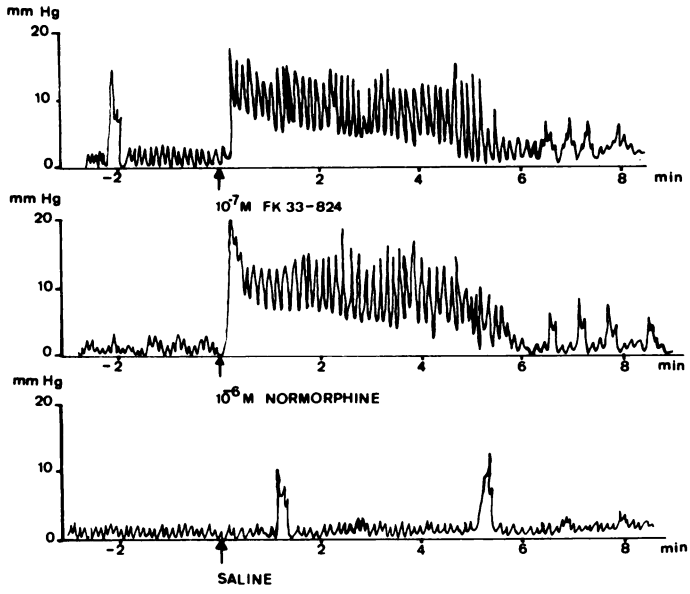

Fig. 2. Motility pattern of the proximal colonic segment before, during and after stimulation with FK 33-824, normorphine or saline.

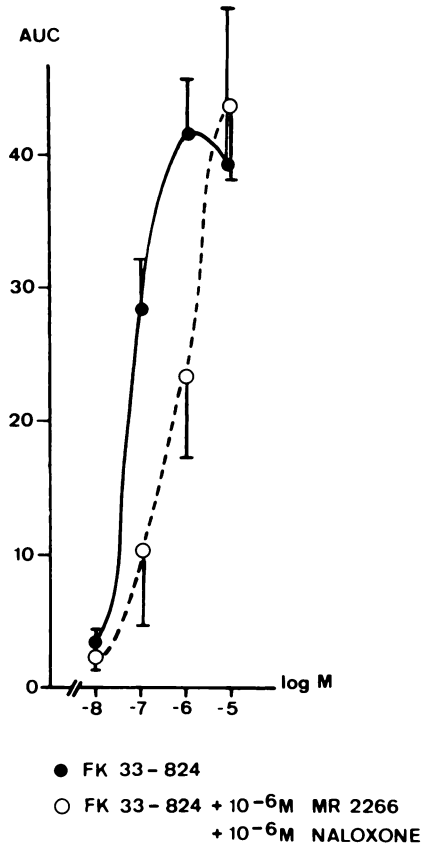

Fig. 4. Dose-response relationship of FK 33-824 without and with a combination of Mr 2266 and naloxone in an equimolar dose on proximal colonic tonic contractions. (AUC, arbitrary units. Mean values ± S.E. of six preparations for each dose level).

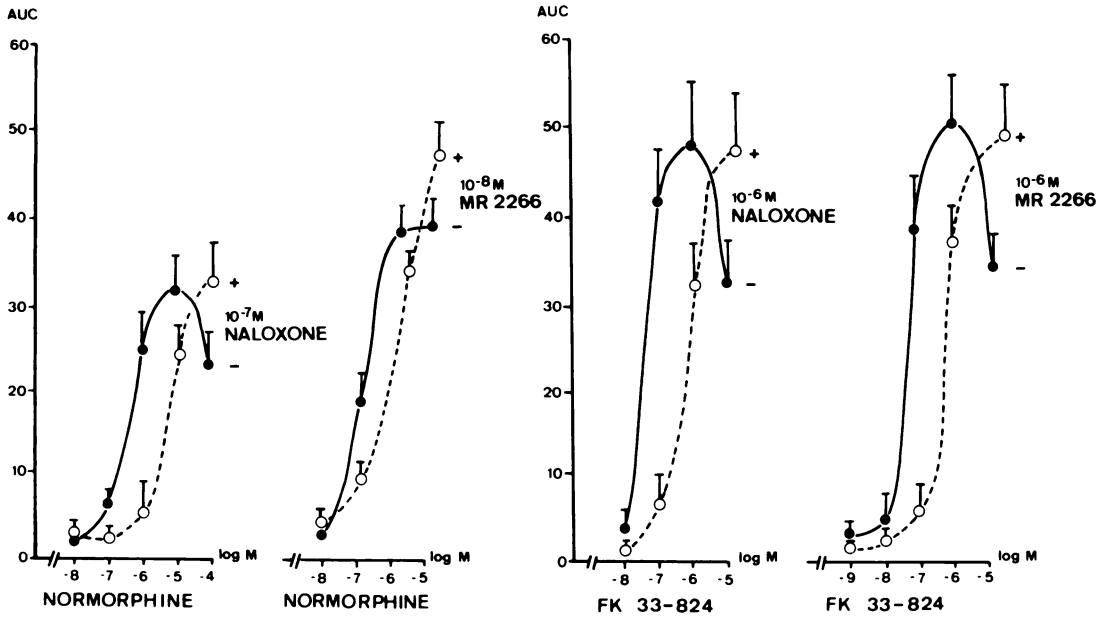

Fig. 3. Dose-response relationship of normorphine or FK 33-824 without and with a given dose of naloxone or Mr 2266 on tonic contraction of the proximal colonic segment (AUC, arbitrary units. Mean values ± S.E. of six preparations for each dose level).

The effect of TTX on the normorphine- and FK 33-824-stimulated tonic contraction of the dissected (isolated colon divided into equal sized sections) proximal and midcolonic segments is illustrated in figure 5.

TTX significantly inhibited the normorphine (t = 5.104, P < .002) and FK 33-824 (t = 3.657, P < .01)-induced tonic contraction in the proximal and enhanced the reaction to FK 33-824 (t = 2.841, P < .02) and normorphine (t = 2.586, P < .02) in the midcolonic segment. The reaction of the distal colonic segment was similar to that of the midcolonic segment.

TTX (10^{-6} M) alone produced a significant tonic contraction at the three colonic sites during the 10 initial min of the 20-min pretreatment period (AUC_{prox} + S.E.: 13.8 ± 2.7, t = 2.91, P < .025; AUC_{mid}: 16.3 ± 3.6, t = 3.55, P < .01; AUC_{dist}: 20.3 ± 3.5, t = 6.84, P < .002). This initial pressure rise was completely suppressed by 10^{-5} M atropine sulfate. After the 20-minute pretreatment of colonic preparations with TTX, no interference with the action of acetylcholine was observed.

Atropine sulfate (10^{-5} M) had no influence on basal motility pattern of colonic preparations and did not affect the FK 33-824- or normorphine-induced tonic contraction at the three sites (all P > .3), but completely abolished the acetylcholine-induced motor response of the colon.

Interaction of isoproterenol and norepinephrine on FK 33-824-or normorphine-stimulated tonic contractions in the proximal colon is given in figure 6. Norepinephrine and isoproterenol reduced basal tone and abolished the phasic contractile activity at the three colonic sites completely. Both substances strongly inhibited FK 33-824- or normorphine-induced tonic contractions. Since the slopes of the dose-response curves without the inhibitor were significantly different from those with the inhibitor (all P < .05), evidence for noncompetitive inhibition was found. Neither 10^{-6} M phentolamine nor 10^{-6} M propranolol interfered with the stimulatory action of FK 33-824 or normorphine, but antagonized the inhibitory action of norepinephrine and isoproterenol, respectively.

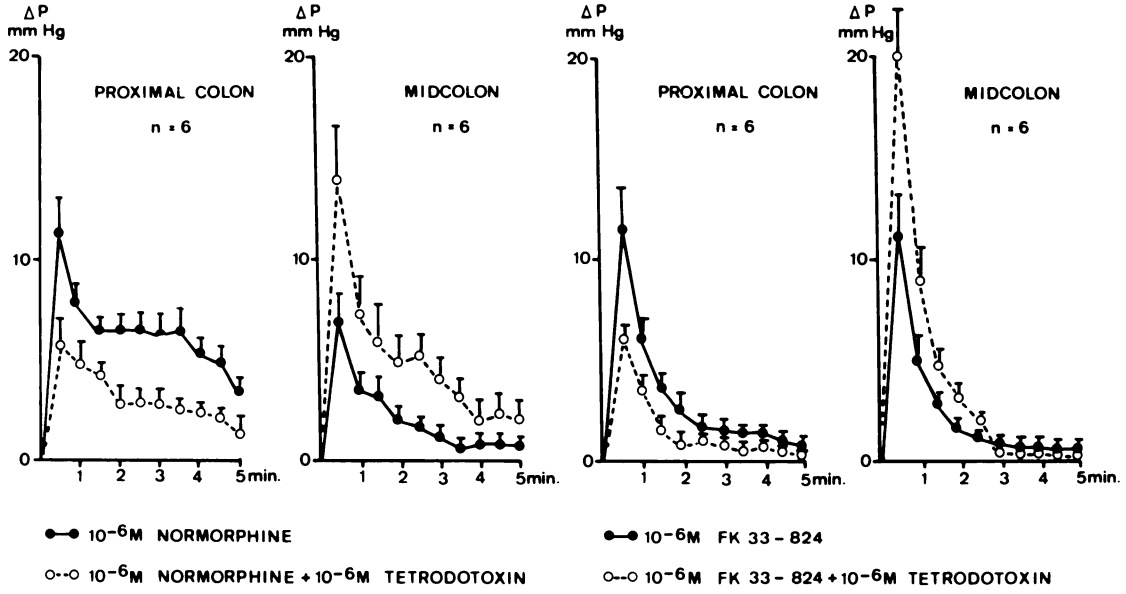

Fig. 5. Interaction of normorphine or FK 33-824 without and with a given dose of TTX on tonic contraction of isolated proximal and midcolonic segment ($\bar{x}$ ± S.E.).

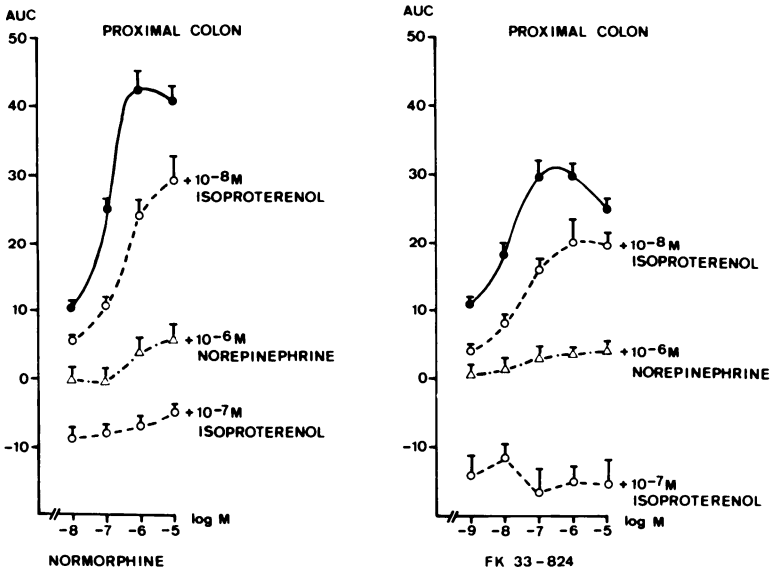

Fig. 6. Dose-response relationship of normorphine or FK 33-824 without and with given doses of norepinephrine or isoproterenol on tonic contraction of the proximal colonic segment (AUC, arbitrary units. $\bar{x}$ ± S.E. of six preparations for each dose level).

The tonic muscular pressure response to FK 33-824 or normorphine was not significantly different from that of a combination with 10^{-6} M methysergide, although methysergide in this concentration inhibited serotonin-induced tonic contraction of colonic muscles substantially.

Discussion

The results of this study demonstrate that the synthetic metenkephalin analog FK 33-824 produces a powerful tonic contraction and increased the strength of phasic contractions for about 6 min followed by a series of high-pressure contractions with a frequency of about 1 to 2 min in the proximal, mid and distal colonic segment of the rat. The pattern of this reaction was similar to that obtained by normorphine. Furthermore, it closely resembled morphine-induced alterations reported from isolated colonic muscle strips of the rat (Gillan and Pollock, 1976).

Further support of an opiate-like action of FK 33-824 on colonic motility resulted from the fact that the putative *mu*-opiate receptor antagonist naloxone competitively inhibited the stimulatory action of FK 33-824 and normorphine by a symmetrical shift of the respective dose-response curves to the right. This type of inhibition indicated the action of FK 33-824 and normorphine on the same receptor. Since Mr 2266, a suggested *kappa*-opiate receptor antagonist (Waterfield and Kosterlitz, 1975; Martin *et al.*, 1976a,b; Gilbert and Martin, 1976; Lord *et al.*, 1977), similarly inhibited the action of FK 33-824 and normorphine on colonic motility as did naloxone, additional evidence of action of the latter two substances on a common receptor was given. Furthermore, the potency ratio of the inhibitory action of naloxone and Mr 2266 on the FK 33-824-stimulated colon was not different and thus also indicative that both opiate receptor antagonists act on the same receptor. These observations are in accordance with the findings of Lord *et al.* (1977) in the guinea-pig ileum in which Mr 2266 also had a potent inhibitory effect on compounds stimulating the *mu*-opiate receptor. Since equimolar doses of both opiate receptor antagonists did not exert a significantly greater inhibition than the single dose opiate receptor antagonist, action of both inhibitors on the same receptor in our preparation is probably. Receptor binding studies are needed to clarify whether Mr 2266 is not a specific *kappa*-opiate receptor antagonist or whether the *kappa*-opiate receptor is identical with the *mu*-opiate receptor in the colon.

Functional evidence for neural opiate receptor sites was found in the undissected colon in which TTX significantly inhibited the stimulatory effects of FK 33-824 or normorphine in all parts. Since no complete inhibition could be achieved even with 10^{-5} M TTX, additional muscular opiate receptor sites must be assumed. Similar conclusions were drawn by Burks (1973) from studies on TTX-morphine interaction on motility of isolated sections of canine intestine.

After dissection of the isolated colon, the effects of FK 33-824 or normorphine and their inhibition by TTX were unchanged in the proximal colonic segment. In the mid and distal segment, however, the inhibitory effect of TTX on FK 33-824 or normorphine stimulation was abolished. This behavior indicates that the neural regulation of colonic motility may be controlled by a neural center located in the proximal colon and that after mechanical interruption of the neural pathways muscular opiate receptor sites may be responsible for the stimulatory action of FK 33-824 or normorphine on colonic tonic contraction and account for this kind of acute "denervation hypersensitivity."

The neural pathway stimulated by FK 33-824 or normorphine seems to be noncholinergic, nonadrenergic and nonserotoninergic in the rat colon, since neither atropine sulfate, propranolol, phentolamine nor methysergide in doses active against the respective agonists in this isolated colon preparation interacted significantly with the effects of FK 33-824 or normorphine at the three colonic sites.

Norepinephrine and isoproterenol substantially inhibited the motility action of FK 33-824 or normorphine in a noncompetitive manner. Since adrenergic receptor agonists produced different effects on colonic motility than FK 33-824 or normorphine, the opiate receptors are not identical with *alpha* or *beta* adrenergic receptors. Furthermore, this finding supports the contention that the neural pathway stimulated by FK 33-824 or normorphine in the rat colon is nonadrenergic.

It is concluded that the met-enkephalin analog FK 33-824 exerts motility actions in the rat colon that are similar to those of normorphine. Both substances act on receptors which have properties of *mu*- and *kappa*-opiate receptors and which are different from *alpha* or *beta* adrenergic receptors. The common FK 33-824-normorphine receptors seem to be located in the intrinsic nervous system and in muscles in the proximal colonic segment and solely in muscular tissue in the mid and distal colonic segment of the rat. The nervous pathway stimulated by FK 33-824 and normorphine is not identified. The data suggest it to be noncholinergic, nonadrenergic and nonserotoninergic.

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