ELSEVIER

European Journal of Pharmacology 314 (1996) 27–32

ejp

Gastric effects of μ-, δ- and κ-opioid receptor agonists on brainstem unitary responses in the neonatal rat

Chun-Su Yuan *

Committee on Clinical Pharmacology and the Department of Anesthesia and Critical Care, The University of Chicago Medical Center, 5841 S. Maryland Avenue, MC 4028, Chicago, IL 60637, USA

Received 11 April 1996; revised 25 June 1996; accepted 28 June 1996

Abstract

Single units in the medial subnucleus of the nucleus tractus solitarii, responding to electrical stimulation of subdiaphragmatic vagal fibers, were recorded extracellularly in an in vitro neonatal rat brainstem-gastric preparation. Selective opioid receptor agonists were applied only to the gastric compartment of the bath chamber and therefore, the brainstem functions of the preparation were not affected. The peripheral gastric effects of the μ-opioid receptor agonist, [D-Ala^2,*N*-MePhe^4,Gly^5-ol]enkephalin (DAMGO) and κ-opioid receptor agonist, {*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methanesulfonate hydrate} (U-50,488H), were evaluated on 69 tonic units that received the subdiaphragmatic vagal input. For approximately 75% of the units observed, DAMGO (1.0 μM; IC_{70}: 80 nM) and U-50,488H (1.0 μM; IC_{70}: 200 nM) induced a concentration-dependent inhibition of 62.7 ± 8.9% (mean ± S.D.) and 50.6 ± 6.2% of the control level of the brainstem neuronal activity, respectively. The μ-opioid selective receptor antagonist, naltrexone and non-selective opioid receptor antagonist, naloxone, respectively, blocked the inhibitory effects by DAMGO and U-50,488H. The δ-opioid receptor agonist, [D-Pen^2,D-Pen^5]enkephalin (DPDPE) (10 μM; IC_{70}: 400 nM) produced a lesser extent of inhibition of 21.9 ± 8.0% in only 10 out of 51 (20%) neurons tested, and this effect was blocked by naloxone. The area of the stomach where gastric opioid receptors contributed most to brainstem unitary activity was also examined. This was achieved by comparing the opioid effects on a whole-stomach preparation to its effects on a partial-stomach preparation. Our data indicated that the distal stomach containing the pylorus played a key role in the gastric effects of μ- and κ-opioid receptors on brainstem neuronal activity. These results suggest that the μ- and κ-opioid receptors of the distal stomach are important in modulation of brainstem neuronal activity and may play a role in regulating the digestive process.

Keywords: Subdiaphragmatic vagus; Nucleus tractus solitarii; Stomach; Opioid receptor; (In vitro); (Neonatal rat)

1. Introduction

Opioid receptors are widely distributed in the central nervous system and throughout the gastrointestinal tract (Manara and Bianchetti, 1985; Hughes et al., 1977). Results from previous studies suggested that opioids can act within the central nervous system to alter autonomic outflow to the gut (Parolaro et al., 1977; Stewart et al., 1978; Galligan and Burks, 1983), and act directly on the gut to change gastrointestinal motility (Daniel et al., 1959; Burks, 1973; Tavani et al., 1980). It seems that an opioid's effects on gastrointestinal motility are due partly to its action in the central nervous system, and partly to its direct effect on the gut.

The inhibitory effect of opioids on gastric emptying and gastrointestinal transit have been observed in both experimental animals and patients (Green, 1959; Kromer, 1989; McCaffrey and Beebe, 1989). Our recent investigation demonstrated that morphine reduced in vitro smooth muscle-strip contractions in guinea-pig ileum and human small intestine, and this inhibition could be blocked by methylnaltrexone, a peripheral opioid receptor antagonist (Yuan et al., 1995). It is possible that this opioid effect on the gut can activate visceral afferent fibers and that these signals are conveyed to certain areas of the central nervous system to modulate the neuronal activities.

The antimotility and antitransit actions could be produced by the administration of opioid receptor agonists into the systemic circulation (Brown and Goldberg, 1985). In cats, intravenous morphine injection reduced the unitary discharges of the caudal brainstem units which received

* Tel.: (1-312) 702-1916; Fax: (1-312) 702-3535; e-mail: cyuan@midway.uchicago.edu

PII S0014-2999(96)00531-6

gastric vagal input (Barber et al., 1988). This inhibitory effect on brainstem neuronal activity could be achieved by activation of opioid receptors on the brainstem cells and/or on the gut. In the present study, an in vitro neonatal rat brainstem-gastric preparation was used to evaluate the peripheral gastric effects of μ-, δ- and κ-opioid receptors on nucleus tractus solitarii neurons that received subdiaphragmatic vagal input. We have also investigated which part of the stomach (proximal vs. distal) played the most important role in the gastric effect of opioid receptor modulation in brainstem unitary responses.

2. Materials and methods

Experiments were performed on Sprague-Dawley neonatal rats of 3–5 days old. After the animal was deeply anesthetized with halothane, a craniotomy was performed and the forebrain was ablated at the caudal border of the pons by transection. The caudal brainstem and cervical spinal cord were isolated by dissection in modified Krebs solution that contained (in mM): NaCl 128.0, KCl 3.0, NaH_2PO_4 0.5, $CaCl_2$ 1.5, $MgSO_4$ 1.0, $NaHCO_3$ 21, mannitol 1.0, glucose 30.0 and *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulphonic acid (Hepes) 10.0. The stomach, connected to the esophagus, with the vagus nerves linking it to the brainstem, was kept and all the other internal organs were removed (see Fig. 1 in Barber et al., 1995). The preparation was then pinned with the dorsal surface upon a layer of Sylgard resin (Dow Corning) in a recording chamber. The preparation was superfused with Krebs solution at 27°C ± 1°C. The bathing solution was aerated continuously with a mixture of 95% O_2 and 5% CO_2 and adjusted to pH 7.35–7.45 (Murakoshi et al., 1985; Smith and Feldman, 1987; Barber et al., 1995).

A suction microelectrode was placed on the right vagal trunk at the gastroesophageal junction for electrical stimulation since only those neurons in the medial subnucleus of the nucleus tractus solitarii receiving subdiaphragmatic vagal input were used in this study. Suction strength was carefully adjusted to keep the nerve in physiological continuity. The nerve fibers were stimulated with single or paired pulses of 200 μA for 0.2 ms at a frequency of 0.5 Hz by a Grass stimulator (model S8800) coupled to a stimulus isolation unit (SIU 5B, Grass Instruments). This current provided a stimulus intensity 1.5–2.0 times that required to produce maximal amplitude of the C-wave in the vagal nerve action potential.

Single unitary activity was recorded extracellularly in the nucleus tractus solitarii by glass microelectrodes filled with 3 M NaCl, which had an impedance of 10–20 MΩ. A collision test was applied to identify orthodromic responses which were used in this study (Lipski, 1981). For histological identification purposes, some glass microelectrodes were filled with 2% pontamine sky blue in 0.5 M sodium acetate solution. After each unitary recording, current was applied at 5 μA in 5 s on/10 s off cycles for approximately 5 min, with the negative lead connected to the microelectrode.

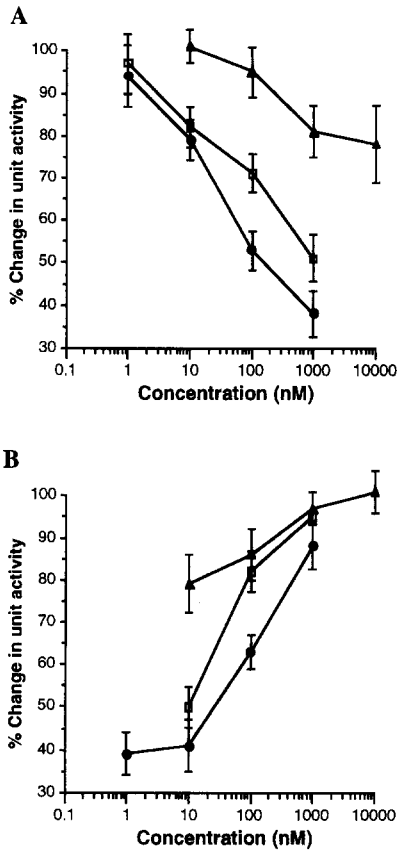

Fig. 1. Concentration-related gastric effects of opioid receptor agonists and antagonists on 52 nucleus tractus solitarii neurons receiving subdiaphragmatic vagal input. (A) The effects of DAMGO (solid circle), U50,488H (open square) and DPDPE (solid triangle). (B) The reversal effects of antagonists, naltrexone (solid circle), naloxone (open square) and naloxone (solid triangle) on DAMGO (80 nM), U50,488H (200 nM) and DPDPE (400 nM) induced inhibition, respectively. Ordinate, discharge rate of nucleus tractus solitarii neurons expressed as percentage of control. The control activity level is normalized to 100%. Abscissa, concentration (nM) of agonists (A) or antagonists in the presence of agonists (B). Brackets indicate the means ± S.E.M.

Opioids could have both peripheral and central actions. To investigate the peripheral gastric effects of opioids on nucleus tractus solitarii units receiving subdiaphragmatic vagal inputs without interfering with central nervous system functions, a partition was made at the mid-thoracic level of the preparation. An agar seal separated the recording bath chamber into a brainstem compartment and a gastric compartment. An incision was performed on the lateral surface of the stomach wall to minimize possible gastric vagal fiber damage. The stomach was opened and its contents removed. The stomach was then pinned down and the mucosa was exposed to the solution of the gastric compartment. This was defined as a whole-stomach preparation. Opioid receptor agonists and antagonists were applied to the gastric compartment and their peripheral gas-

tric effects on the brainstem neuronal activity were evaluated. After each observation, drug(s) were washed out from the gastric compartment. The nucleus tractus solitarii neuronal responses observed during pre-trial (control) were compared to post-trial (washout), to confirm that brainstem neuronal activity had returned to the control level after washout. Tachyphylaxis was not evident in our experimental conditions since response to reapplication of a given concentration of agonist varied by less than 5%.

The drugs used were [D-Ala^2,*N*-MePhe^4,Gly^5-ol]enkephalin or DAMGO (Sigma); {trans-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methanesulfonate hydrate} or U-50,488H (Upjohn); [D-Pen^2,D-Pen^5]enkephalin or DPDPE (Sigma); naltrexone (Sigma); naloxone (Sigma).

During each experiment nucleus tractus solitarii unitary discharges were amplified with high gain AC-coupled amplifiers, displayed on a storage oscilloscope and recorded on a tape recorder. The data from the brainstem unitary activity was analyzed on the basis of action potential discharge rate and drug concentration-related effects. The number of action potentials in a certain duration were measured under pre-trial, trial and post-trial conditions. The control data (pre-trial) were normalized to 100%, and the brainstem neuronal activity during and after trials was compared to the control data. Results were analyzed using Student's *t*-test and Mann-Whitney *U*-test with $P < 0.05$ considered statistically significant.

3. Results

3.1. Peripheral gastric effects of DAMGO, U-50,488H and DPDPE on nucleus tractus solitarii unitary activity

Sixty-nine tonic units receiving subdiaphragmatic vagal input were recorded in the nucleus tractus solitarii in the neonatal rat brainstem-gastric preparation. The peripheral gastric effects of selective opioid receptor agonists were evaluated and recording locations in the nucleus tractus solitarii were identified histologically. When a μ-opioid receptor agonist, DAMGO, and a κ-opioid receptor agonist, U-50,488H, were applied to the gastric compartment, the firing rate in the majority of the nucleus tractus solitarii neurons was decreased in a concentration-related fashion. In 52 out of 69 units observed, DAMGO (1.0 μM; IC_{70}: 80 nM) produced an inhibitory effect of 62.7 ± 8.9% (mean ± S.D.) of the control level of the brainstem neuronal activity (Fig. 1A). In 49 out of 67 units observed, U-50,488H (1.0 μM; IC_{70}: 200 nM) induced 50.6 ± 6.2% (mean ± S.D.) inhibition of the nucleus tractus solitarii unitary activity (Fig. 1A). The μ-opioid receptor selective antagonist, naltrexone, and the non-selective opioid receptor antagonist, naloxone, respectively, blocked these inhibitory effects by DAMGO (80 nM) and U-50,488H (200 nM) (Fig. 1B). The remaining brainstem cells (approximately 25% recorded) showed no response to these two opioid receptor agonists.

The δ-opioid receptor agonist, [D-Pen^2,D-Pen^5]enkephalin (DPDPE) (10 μM; IC_{70}: 400 nM) produced a lesser extent of concentration-dependent inhibition of 21.9 ± 8.0% in only 10 out of 51 neurons tested, and this effect of DPDPE (400 nM) was blocked by naloxone (Fig. 1A,B). Naltrexone did not block the effect of DPDPE (10 μM).

Some of these results are summarized in Table 1.

Application of the opioid receptor antagonists alone did not show significant changes in nucleus tractus solitarii unitary activity.

3.2. Distribution of gastric opioid receptors affecting nucleus tractus solitarii unitary activity

To investigate the distribution of the gastric opioid receptors that affect subdiaphragmatic vagally evoked nucleus tractus solitarii unitary responses, a whole-stomach preparation and a partial-stomach preparation were used. The gastric mucosa structure of the proximal and the distal stomach under the dissecting scope appear distinctly different. This mucosa structure difference was used as a landmark to make the partial-stomach preparation. Peripheral gastric effects of opioid receptor agonists were observed first in the whole-stomach preparation. Next, the proximal part or the distal part (containing the pylorus) of the stomach was carefully removed, while unitary recording in the nucleus tractus solitarii was maintained. Opioid receptor agonist effects on the same nucleus tractus solitarii cell were then observed in the proximal-stomach or distal-stomach preparations.

Twenty-three nucleus tractus solitarii units that responded to DAMGO (1.0 μM) and 26 nucleus tractus solitarii units that responded to U-50,488H (1.0 μM) were

Table 1
Gastric inhibitory effects of opioid receptor agonists, DAMGO (μ-opioid receptor agonist), U50,488H (κ-opioid receptor agonist) and DPDPE (δ-opioid receptor agonist) on nucleus tractus solitarii unitary activity

Drug	Number of units	Number of inhibitory responses	% response	% inhibition (mean ± S.D.)
DAMGO (1.0 μM)	69	52	75.4	62.7 ± 8.9
U50,488H (1.0 μM)	67	49	73.1	50.6 ± 6.2
DPDPE (10 μM)	51	10	19.6	21.9 ± 8.0

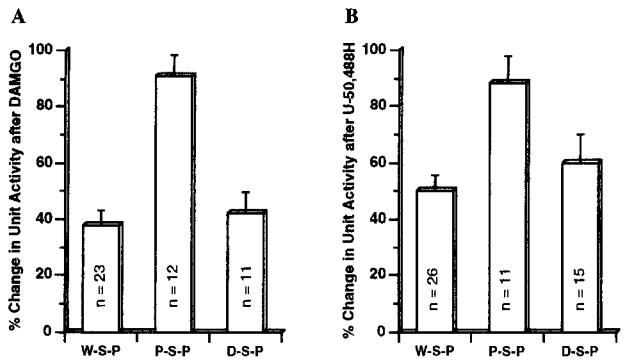

Fig. 2. Peripheral gastric effects of DAMGO and U50,488H on nucleus tractus solitarii neuronal activity in the whole-stomach preparation vs. the partial-stomach preparation. (A) The effect of DAMGO (1.0 μM) and (B) the effects of U50,488H (1.0 μM). The control activity level is normalized to 100%. There is a significant difference between the whole-stomach preparation (W-S-P) and proximal-stomach preparation (P-S-P) in both DAMGO and U50,488H groups (both $P < 0.001$). There is a significant difference between the proximal-stomach preparation (P-S-P) and distal-stomach preparation (D-S-P) in both DAMGO and U50,488H groups ($P < 0.001$ and $P < 0.01$ respectively). However, there is no significant difference between the whole-stomach preparation (W-S-P) and the distal-stomach preparation (D-S-P) in either DAMGO or U50,488H groups.

observed in both the whole-stomach and partial-stomach preparations (Fig. 2). There was a significant difference between the gastric effects of DAMGO and U-50,488H on the whole-stomach and proximal-stomach preparations in the inhibitory brainstem neuronal responses. There was a significant difference between the gastric effects of these two opioid receptor agonists on the proximal-stomach and distal-stomach preparations. However, there was no significant difference between the whole-stomach and distal-stomach preparations. These results suggest that the distal stomach containing the pylorus plays an important role in the gastric effects of μ- and κ-opioid receptor agonists on subdiaphragmatic vagally evoked brainstem unitary responses.

4. Discussion

The present study employed an in vitro neonatal rat brainstem-gastric preparation initially developed at the University of Arizona (Barber et al., 1995) to investigate the peripheral gastric effects of selective opioid receptor agonists on nucleus tractus solitarii unitary activity. The advantageous feature of this preparation is the ability to change the local environment in the gastric compartment without interfering with any brainstem functions, while mimicking an in vivo preparation in which the physiological inputs can be identified. It was the nature of this preparation that permitted the examination of opioid gastric effects on the subdiaphragmatic vagally evoked brainstem neurons observed in this study.

The effect of opioids on gastrointestinal motility and transit is appreciated as a clinical phenomenon. Immunoreactive studies have demonstrated that μ-, δ- and κ-opioid receptors have a widespread distribution in the gastrointestinal tract (Burks et al., 1988; Spampinato et al., 1988), and many attempts have been made to differentiate those opioid receptors responsible for the inhibition of gastrointestinal motility and transit. Pharmacological investigations have shown that μ-opioid receptor agonists inhibit gastric and intestinal transit (Shook et al., 1987). The δ-opioid receptor agonists have been observed to decrease gastric emptying (Shea-Donohue et al., 1983) as well as gastric and intestinal motility (Bueno et al., 1985). However, more selective δ-opioid receptor agonists had no effect on gastric emptying or intestinal transit (Shook et al., 1987; Tavani et al., 1990). The κ-opioid receptor agonists have also been demonstrated to suppress the gastrointestinal transit (Ward and Takemori, 1983), but later studies have revealed inconsistent results in different animals (Shook et al., 1987; Culpepper-Morgan et al., 1988; Tavani et al., 1990). Results from the study of opioid effects on the contractility of dispersed gastric smooth muscle cells in guinea-pig demonstrated that these cells possess μ- and κ-opioid receptors but not δ-receptors (Zhang et al., 1992). It seems likely, therefore, that different types of opioid receptors have different effects on gastrointestinal motility and transit. Understanding the pharmacological effects of opioids on gastrointestinal motility is further complicated by difference of effect among species, the region of the gastrointestinal tract examined, and the precise experimental conditions.

The present study was designed to examine the gastric effects of μ-, δ- and κ-opioid receptor agonists on central autonomic processing. The nucleus tractus solitarii is the first central autonomic processing station, and a substantial number of cells responding to electrical stimulation of the gastric vagal branches have been recorded in the nucleus tractus solitarii in previous experiments on the cat (Yuan and Barber, 1990). The aim of this study was to test peripheral gastric effects of opioid receptor agonists on brainstem neuronal activity in neonatal rats.

In this study, we observed that the peripheral gastric application of μ-, δ- and κ-opioid receptor agonists, to different extents, decreased excitability in nucleus tractus solitarii neuronal activity in a dose-related fashion. Results from previous studies showed that morphine reduced the stimulation-elicited gastric response dose-dependently and vagal afferent fibers were responsible for this inhibition (Kurahashi et al., 1983; Okamoto et al., 1986). It appears that opioid receptor agonists in the gastric compartment of our preparation activate gastric opioid receptors which in turn modulate action potentials in vagal afferent fibers. The nerve action potentials are conveyed by the vagus nerve to nucleus tractus solitarii neurons. These gastric afferents subsequently decrease excitability of the brainstem neurons and these neurons in turn contact different areas in the central nervous system causing a decrease in gastric motility and ultimately inhibiting gastric emptying.

However, in our experimental conditions, we were not able to differentiate whether brainstem neuronal responses to gastric opioid receptor agonist application were mediated through the activation of opioid receptors directly on vagal afferents or were secondary to activation of receptors on gastric smooth muscle fibers.

Results from this study, utilizing the whole-stomach and the partial-stomach preparations, demonstrated that the distal stomach, not the proximal stomach, is important in the opioid gastric effects on nucleus tractus solitarii neuronal activity. Daniel (1966) showed that morphine inhibited gastric emptying and suggested that antral spasm could account for delayed gastric emptying. It has also been revealed that, in the stomach, morphine increases the amplitude and decreases the frequency of antral contractions (Konturek, 1980). Cholecystokinin, an important gastrointestinal neuropeptide which also slows gastric emptying, has its binding sites located in the pyloric sphincter, the antral circular muscle and the gastric mucosa at different stages of development (Robinson et al., 1987). Using the same preparation as in this study, Yuan and Barber (1993) showed that the distal stomach is important in cholecystokinin effects on brainstem neuronal activity. It is possible that the opioid-binding sites are also distributed differently in the stomach of the neonatal rat, and this speculation needs to be confirmed in future experiments.

In summary, an in vitro neonatal rat brainstem-gastric preparation was used to evaluate the peripheral gastric effects of selective opioid receptor agonists on nucleus tractus solitarii neurons that responded to electrical stimulation of the subdiaphragmatic vagal fibers. Our results suggest that the μ- and κ-opioid receptors of the distal stomach play an important role in modulation of brainstem neuronal activity and may play a role in regulating the digestive process.

Acknowledgements

The author wishes to thank Tasha Lowell for her technical assistance.

References

Barber, W.D., C.S. Yuan and T.F. Burks, 1988, Morphine inhibition of brainstem responses to gastric vagal input, Gastroenterology 94, A21.

Barber, W.D., C.S. Yuan, T.F. Burks, J.L. Feldman and J.J. Greer, 1995, In vitro brainstem-gastric preparation with intact vagi for study of primary visceral afferent input to dorsal vagal complex in caudal medulla, J. Auton. Nerv. Syst. 51, 181.

Brown, D.R. and L.I. Goldberg, 1985, The use of quaternary narcotic antagonists in opiate research, Neuropharmacology 24,181.

Bueno, L., J. Fioramonti, C. Honde, M.J. Fargeas and M.P. Primi, 1985, Central and peripheral control of gastrointestinal and colonic motility by endogenous opiates in conscious dogs, Gastroenterology 88, 549.

Burks, T.F., 1973, Mediation by 5-hydroxytryptamine of morphine stimulant actions in dog intestine, J. Pharmacol. Exp. Ther. 185, 530.

Burks, T.F., D.A. Fox, L.D. Hirning, J.E. Shook and F. Porreca, 1988, Regulation of gastrointestinal function by multiple opioid receptors, Life Sci. 43, 2177.

Culpepper-Morgan, J.A., M.J. Kreek, P.R. Holt, D. LaRoche, J. Zhang and L. O'Bryan, 1988, Orally administered κ as well as μ opioid agonists delay gastrointestinal transit time in the guinea pig, Life Sci. 42, 2073.

Daniel, E.E. 1966, Further studies of the pharmacology of the pyloric region. Analysis of the effects of intra-arterial histamine, serotonin, phenylbiguanide, morphine and other drugs on the antrum and duodenal bulb, Can. J. Physiol. Pharmacol. 44, 981.

Daniel, E.E., W.H. Sutherland and A. Bogoch, 1959, Effects of morphine and other drugs on motility of the terminal ileum, Gastroenterology 36, 510.

Galligan, J.J. and T.F. Burks, 1983, Centrally mediated inhibition of small intestinal transit and motility by morphine in the rat, J. Pharmacol. Exp. Ther. 226, 356.

Green, A.F., 1959, Comparative effects of analgesics on pain threshold, respiratory frequency and gastrointestinal propulsion, Br. J. Pharmacol. 14, 34.

Hughes, J., H.W. Kosterlitz and T.W. Smith, 1977, The distribution of methionine-enkephalin and leucine-enkephalin in the brain and peripheral tissues, Br. J. Pharmacol. 61, 639.

Konturek, S.J., 1980, Opiates and the gastrointestinal tract, Am. J. Gastroenterol. 74, 285.

Kromer, W., 1989, The current status of opioid research on gastrointestinal motility, Life Sci. 44, 579.

Kurahashi, K., M. Fujiwara and T. Taniguchi, 1983, Cholinergic excitatory response of stomach to stimulation of the vagal trunk in cats with chronic supra-nodose vagatomy, IRCS Med. Sci. 11, 62.

Lipski, J., 1981, Antidromic activation of neurones as an analytic tool in the study of the central nervous system, J. Neurosci. Meth. 4, 1.

Manara, L. and A. Bianchetti, 1985, The central and peripheral influences of opioids on gastrointestinal propulsion, Ann. Rev. Pharmacol. Toxicol. 25, 249.

McCaffrey, M. and A. Beebe, 1989, Managing your patients' adverse reactions to narcotics, Nursing 19, 166.

Murakoshi, T., T. Suzue and S. Tamai, 1985, A pharmacological study on respiratory rhythm in the isolated brainstem-spinal cord preparation of the newborn rat, Br. J. Pharmacol. 86, 95.

Okamoto, T., K. Kurahashi, T. Tsubomura and M. Fujiwara, 1986, Effects of morphine on hexamethonium-sensitive and resistant excitatory responses of stomach to stimulation of vagal trunk in cats, Life Sci. 39, 31.

Parolaro, D., M. Sala and E. Gori, 1977, Effect of intracerebroventricular administration of morphine upon intestinal motility in rat and its antagonism with naloxone, Eur. J. Pharmacol. 46, 329.

Robinson, P.H., T.H. Moran, M. Goldrich and P.R. McHugh, 1987, Development of cholecystokinin binding sites in rat upper gastrointestinal tract, Am. J. Physiol. 252, G529.

Shea-Donohue, P.T., N. Adams, J. Arnold and A. Dubois, 1983, Effects of metenkephalin and naloxone on gastric emptying and secretion in rhesus monkeys, Am. J. Physiol. 245, G196.

Shook, J.E., J.T. Pelton, V.J. Hruby and T.F. Burks, 1987, Peptide opioid antagonist separates peripheral and central opiate antitransit effects, J. Pharmacol. Exp. Ther. 243, 492.

Smith, J.C. and J.L. Feldman, 1987, In vitro brainstem-spinal cord preparations for study of motor systems for mammalian respiration and locomotion, Neurosci. Meth. 21, 321.

Spampinato, S., G.L. Feri, S. Candeletti, P. Romauldi, E, Cavicchini, G. Soimero, G. Labo and S. Ferri, 1988, Regional distribution of immunoreactive dynorphin A in the human gastrointestinal tract, Neuropeptides 11, 101.

Stewart, J.J., N.W. Weisbrodt and T.F. Burks, 1978, Central and peripheral actions of morphine on intestinal transit, J. Pharmarcol. Exp. Ther. 205, 547.

Tavani, A., G. Bianchi, P. Ferretti and L. Manara, 1980, Morphine is

most effective by intraperitoneal route: Evidence for local action, Life Sci. 27, 2211.

Tavani, A., P. Petrillo, A. La Regina and M. Sbacchi, 1990, Role of peripheral μ, δ and κ opioid receptors in opioid-induced inhibition of gastrointestinal transit in rats, J. Pharmacol. Exp. Ther. 254, 91.

Ward, S.J. and A.E. Takemori, 1983, Relative involvement of receptor subtypes in opioid-induced inhibition of gastrointestinal transit in mice, J. Pharmacol. Exp. Ther. 224, 359.

Yuan, C.S. and W.D. Barber, 1990, Brainstem evoked response to dorsal vagal gastric input from the proximal stomach, J. Auton. Nerv. Syst. 31, 67.

Yuan, C.S. and W.D. Barber, 1993, Peripheral gastric effects of CCK-8 on brainstem unitary responses in the in vitro neonatal rat, Soc. Neurosci. Abstr. 19, 1693.

Yuan, C.S., J.F. Foss and J. Moss, 1995, Effects of methylnaltrexone on morphine-induced inhibition of contraction in isolated guinea-pig ileum and human intestine, Eur. J. Pharmacol. 276, 107.

L. Zhang, Z.F. Gu, T. Pradhan, R.T. Jensen and P.N. Maton, 1992, Characterization of opioid receptors on smooth muscle cells from guinea pig stomach. Am. J. Physiol. 262, G461.