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Identification and Localization of Opioid Receptors in the Opossum Lower Esophageal Sphincter[1]

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ABSTRACT

Effects of different opioid receptor agonists, buprenorphine, ketocyclazocine, N-allylnormetazocine, (D-Ala^2, D-Leu^5)-enkephalin (DADL) and meperidine were investigated on the lower esophageal sphincter (LES) in anesthetized opossums. The LES pressures were monitored with a continuously perfused assembly of catheters which was anchored in the sphincter. The opioid agonists were administered i.a. in the esophageal branch of the left gastric artery. Buprenorphine, ketocyclazocine and meperidine caused dose-dependent reductions in the LES pressures. The effect of the maximally effective dose of buprenorphine was antagonized by naloxone (400 μg/kg i.v.). However, the inhibitory effects of the maximally effective doses of ketocyclazocine and meperidine required a larger dose of naloxone (4 mg/kg) for antagonism. Tachyphylaxis to buprenorphine, ketocyclazocine and meperidine showed no cross-tachyphylaxis used in their maximally effective doses. Tetrodotoxin, atropine, hexamethonium, propranolol, haloperidol and indomethacin did not modify the inhibitory effect of buprenorphine or ketocyclazocine. The fall in LES pressures with meperidine was, however, significantly antagonized by tetrodotoxin ($P < .05$). N-allylnormetazocine and DADL caused dose-related contraction of the LES. Their effects were not modified by 400 μg/kg of naloxone but were antagonized by larger doses of naloxone (4 mg/kg). Tachyphylaxis to DADL failed to modify the excitatory response of N-allylnormetazocine. Tetrodotoxin and atropine antagonized the excitatory effect of N-allylnormetazocine but not that of DADL. These studies suggest that: 1) *mu*, *kappa*, *sigma* and *delta* opioid receptors are present in the LES; 2) *mu* and *kappa* receptors are present on the sphincter muscle, and their activation causes inhibition of LES tone; 3) *sigma* receptor activation causes LES contraction by activation of cholinergic neurons; 4) activation of *delta* receptors, which may lie on the sphincter muscle, causes LES contraction; and 5) meperidine may activate yet another opioid receptor, present on the nonadrenergic inhibitory neurons, causing inhibition of the sphincter.

Morphine and other opioids exert many potent effects on gastrointestinal secretions and motility (Jaffe and Martin, 1980; Ambinder and Schuster, 1979; Konturek, 1980). Some of these effects are centrally mediated, whereas others are exerted at the peripheral sites in the gut (Stewart *et al.*, 1978; Schulz *et al.*, 1979).

Martin *et al.* (1976) suggested the existence of separate *mu*, *kappa* and *sigma* opioid receptors based upon differing pharmacological effects of certain opioid agonists. Further studies also identified the *delta* opioid receptor (Lord *et al.*, 1977). Heterogeneity of opioid receptors is also supported by tachyphylaxis and cross-tachyphylaxis experiments, and differential sensitivity to antagonism by naloxone in physiological and binding studies (Lord *et al.*, 1977; Snyder and Goodman, 1980; Schulz *et al.*, 1980; Cowan *et al.*, 1979).

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The opossum lower esophageal sphincter is composed of smooth muscle fibers and maintains a continuous tonic contraction at rest. Its physiology and pharmacology has been extensively investigated (Goyal and Rattan, 1978). The sphincter is innervated by noncholinergic, nonadrenergic inhibitory neurons and cholinergic excitatory neurons (Goyal and Cobb, 1981). Both these neuron types are present intramurally. The noncholinergic, nonadrenergic inhibitory neurons receive preganglionic input from fibers carried in the vagus nerve (Goyal and Rattan, 1975). The synaptic transmission involves both nicotinic and muscarinic receptors (Goyal and Rattan, 1975). The cholinergic excitatory neurons receive input from preganglionic cholinergic neurons which are carried in the sympathetic pathways (Fournet *et al.*, 1979).

Adrenergic nerves exert excitatory effect *via* the *alpha* adrenergic receptors. *Beta* adrenergic receptors mediate inhibition of LES (Goyal and Rattan, 1978).

It has been shown that the resting tone of LES is largely due to the intrinsic myogenic property of the muscle and the action of excitatory and inhibitory neurohumoral agents modulate the resting myogenic tone (Goyal and Rattan, 1978; Goyal and

ABBREVIATIONS: LES, lower esophageal sphincter; DADL, (D-Ala^2, D-Leu^5)-enkephalin; LESP, LES pressures.

Cobb, 1981). Because of this arrangement, direct or indirect excitatory and inhibitory effects of drugs on the sphincter are easily identifiable. The arterial supply of the LES is also well defined so that drugs can be administered i.a. to minimize distant effects (Goyal and Rattan, 1973).

We performed experiments to: 1) investigate the effects of activation of different opioid receptors by their selective agonists on the LES; 2) characterize these receptors by investigating tachyphylaxis and cross-tachyphylaxis and the effect of different doses of naloxone; 3) localize these receptors to muscle or neural sites by investigating the influence of tetrodotoxin and other antagonists or agents which are known to modify LES activity (Goyal and Rattan, 1978).

We used buprenorphine, ketocyclazocine, N-allylnormetazocine (SKF-10,047) and DADL as prototype agonists of *mu*, *kappa*, *sigma* and *delta* receptors, respectively. We also investigated meperidine because it is one of the most commonly used opioids in clinical practice and also because the opioid receptor activated by it is not clearly defined (Gilbert and Martin, 1976; Cowan *et al.*, 1979).

Methods

The studies were performed in 27 adult opossums of either sex. The animals weighed between 1.9 and 3.5 kg. The animals were anesthetized with i.p. pentobarbital (40 mg/kg). After anesthesia, the animals were strapped supine on the animal board. The brachial vein was cannulated for i.v. administration of substances and the brachial artery was cannulated for continuous recording of blood pressure.

The respiration was assisted with an artificial respirator (model 661; Harvard Apparatus Co., Inc., Millis, MA) through the endotracheal tube. The body temperature of the animals was maintained at 35.5°C with a water-circulating heating pad (model K-1-3; Borman-Rupp Industries Div., Bellville, OH). The vagus nerves were isolated in the cervical region and severed.

A continuously perfused, low compliance, specially designed catheter assembly of four polyvinyl catheters was used to record pressures from the LES. The details of the catheter assembly and the technique of the perfusion system have been described before (Goyal and Rattan, 1976).

Intraluminal sphincter pressures in relation to atmospheric pressure were recorded on a Beckman dynograph recorder (model R612; Beckman Instruments, Inc., Schiller Park, IL) using Statham pressure transducers (model P23Db; Statham Medical Instruments, Oxnard, CA). Once the high-pressure zone of the LES was identified manometrically, the recording catheter assembly was anchored inside the LES as described previously (Goyal and Rattan, 1976). This arrangement minimized axial movement of the catheters in relation to the LES during the experiment.

The lower esophageal sphincter pressure readings were taken from the tops of the phasic variations. The sphincter pressure after administration of drugs represented the maximal deviations (increase or decrease) in the LES pressure over a 5-min period after treatment.

The esophageal branch of the left gastric artery to the LES was cannulated for i.a. administration of different opioids. The gastric contents were continuously syphoned out with an intragastric catheter.

The following agents were used: atropine sulfate (Eli Lilly and Company, Indianapolis, IN); buprenorphine hydrochloride (a gift from Dr. A. Cowan of the Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA); DADL (Peninsula Laboratories, Inc., San Carlos, CA); haloperidol (McNeil Laboratories, Inc., Fort Washington, PA); hexamethonium hydrochloride (City Chemical Corporation, New York, NY); indomethacin (Sigma Chemical Co., St. Louis, MO); ketocyclazocine methosulfonate (a gift from Dr. A. E. Soria of Sterling-Winthrop Research Institute, Rensselaer, NY); meperidine hydrochloride (Winthrop Laboratories, Inc., New York, NY); methysergide maleate (a gift from Sandoz Pharmaceuticals, East Hanover, NJ); N-allylnormetazocine or SKF-10,047 (a gift from Dr. E. L. May of the Department of Pharmacology, Medical College of Virginia, Richmond, VA); naloxone hydrochloride (a gift from C. A. Segretta of Endo Laboratories, Inc., Garden City, NY); propranolol hydrochloride (Ayerst Laboratories, New York, NY); pyrilamine maleate (a gift from Dr. M. E. Garabedian of Alcon Laboratories, Inc., Fort Worth, TX); and tetrodotoxin (Calbiochem, San Diego, CA).

Different agents were dissolved or diluted in physiological saline. Indomethacin was dissolved in 50 mM Tris-HCl buffer (pH 8), and the final concentration of the stock solution was 5 mg/ml.

The doses of different drugs which have salts were calculated on the basis of their salts. Different opioids were administered i.a. in the branch of the left gastric artery over a 10-sec period. The volumes of i.a. boluses ranged from 0.15 to 0.3 ml, whereas those given i.v. varied from 0.5 to 2 ml. The administration of physiological saline (used as a solvent or diluent of different agents) in these volumes by either of the routes had no effect on the LES. After injections, the cannulas were flushed with 0.4 ml of physiological saline i.a. or 1 ml i.v.

Different antagonists were given i.v. Their doses were as follows: atropine (30 μg/kg); hexamethonium (20 mg/kg); indomethacin (5 mg/kg); phentolamine (1 mg/kg); propranolol (1 mg/kg); pyrilamine or haloperidol (3 mg/kg); and methysergide (200 μg/kg). We have shown previously that these doses of the antagonists are able to antagonize significantly the maximal responses of their respective agonists given in the branch of the left gastric artery (Rattan and Goyal, 1979). Tetrodotoxin was administered in doses of 5 μg/kg, 30 min apart as needed to abolish the response of the esophageal balloon distention in causing LES relaxation as described previously (Goyal and Rattan, 1976).

Doses of naloxone to be used were determined by monitoring the effects of various doses of naloxone on the response of the LES to maximal doses of buprenorphine and meperidine. Buprenorphine (0.25 μg/kg) and meperidine (40 μg/kg) in the absence of naloxone caused a fall in LESP of 84.3 ± 6.3 and 77.8 ± 2.8%, respectively. In the presence of 200, 400, 1000, 2000 and 4000 μg/kg of naloxone, the percentage fall in LESP with buprenorphine was 40.4 ± 6.7, 9.0 ± 2.3, 8.4 ± 0.9, 6.0 ± 0.3 and 13.3 ± 3.8%, respectively, whereas with meperidine, it was 77.6 ± 4.3, 79.7 ± 0.9, 76.2 ± 7.6, 56.2 ± 7.8 and 9.6 ± 1.5%, respectively. The smallest dose of naloxone which caused maximal antagonism of buprenorphine was 400 μg/kg and in the case of meperidine it was 4 mg/kg. Naloxone in the dose of 4 mg/kg failed to modify the effect of unrelated substances like isoproterenol and bethanechol on LESP. Isoproterenol caused 85.5 ± 6.2% fall and bethanechol caused 107.5 ± 5.5% rise in LESP control experiments, and in the presence of 4 mg/kg of naloxone, these values were 83.8 ± 2.7% fall and 109.6 ± 5.4% rise, respectively. The doses of 400 μg/kg and 4 mg/kg i.v. of naloxone were used in the rest of the studies.

Tachyphylaxis to buprenorphine, ketocyclazocine and meperidine was achieved by administering 100 times the maximal effective dose of the opioid in a single bolus. Tachyphylaxis to DADL was achieved by continuous i.a. infusion of the opioid in the dose of 2.5 μg/kg/min. The continuous infusion was used because of rapid metabolism of DADL in the body.

The resting sphincter pressures before treatment and the change in pressure after the treatment were determined in millimeters of mercury and expressed as mean ± S.E. for each group of studies. The statistical analysis was performed using the paired *t* test. The changes in pressures were also expressed as percentages.

Results

Effects of opioid agonists on LES. Examples of the effects of i.a. injections of buprenorphine, ketocyclazocine, meperidine, N-allylnormetazocine and DADL are shown in figure 1. Buprenorphine, ketocyclazocine and meperidine caused a fall in LESP. In contrast, N-allylnormetazocine and DADL caused an increase in LESP.

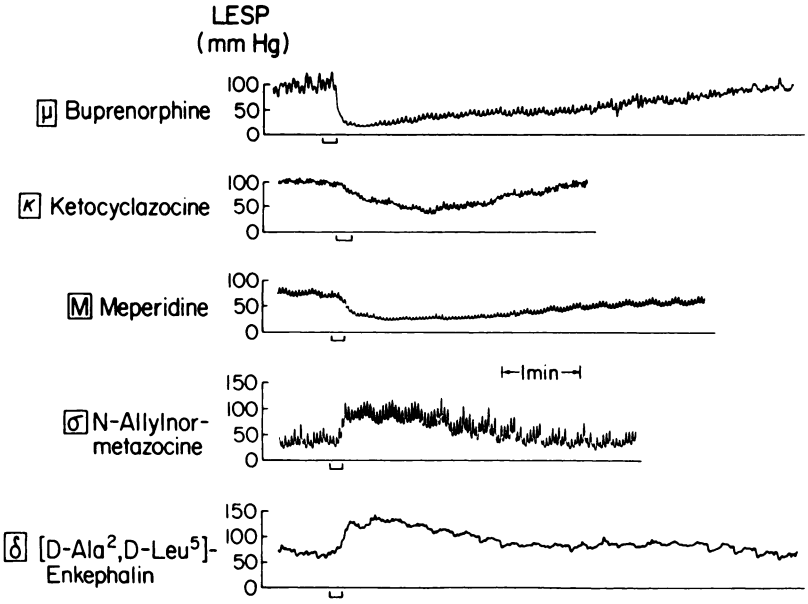

Fig. 1. Examples of effects of different opioids administered in the branch of the left gastric artery on the lower esophageal sphincter. Buprenorphine, ketocyclazocine and meperidine caused a fall in LESP, whereas N-allylnormetazocine and DADL caused increase in LESP.

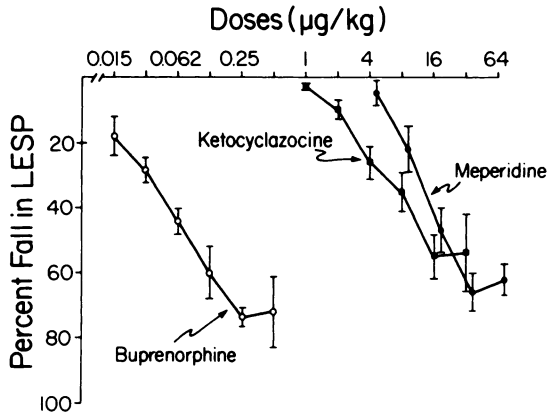

Fig. 2. Comparison of dose-response curves to the inhibitory effect of buprenorphine hydrochloride (MW 504), ketocyclazocine methosulfonate (MW 381.5) and meperidine hydrochloride (MW 283.79). Different doses are represented on the log scale on horizontal axes. Each point represents the mean ± S.E. of four to five observations. On molar basis buprenorphine was 100 and 500 times more potent than ketocyclazocine and meperidine, respectively.

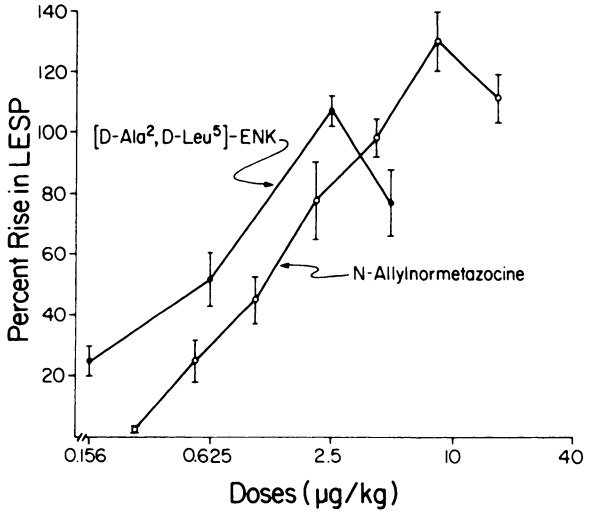

Fig. 3. Dose-response curves showing the excitatory effect of (D-Ala², D-Leu⁵)-enkephalin (ENK) (MW 569.73) and N-allylnormetazocine (MW 293.84) on the LESP. Different doses are expressed on horizontal axes on the log scale. Note that both substances caused a dose-dependent rise in LESP. Each point is the mean ± S.E. of four to six observations. On a molar basis DADL was 4 times as potent as N-allylnormetazocine.

The doses that produced maximal responses in the LES when administered i.a. failed to elicit any significant effect on the LES when administered i.v.

Figure 2 summarizes the dose-response curves of the effects of buprenorphine, ketocyclazocine and meperidine on the LESP. All these agents caused dose-related decrease in the LESP. The maximal inhibition was different for different opioids. The maximally effective doses of buprenorphine, ketocyclazocine and meperidine were 0.25, 16 and 40 μg/kg, respectively. On the molar basis, at ED_{50} levels, buprenorphine was approximately 100 and 500 times more potent than ketocyclazocine or meperidine, respectively.

Figure 3 summarizes the dose-response curves of the effects of N-allylnormetazocine and DADL on the LESP. Both of these agents cause dose-dependent increases in the sphincter pressure. The maximal effective doses of N-allylnormetazocine and DADL were 8 and 2.5 μg/kg, respectively. Supramaximal doses of these agents showed reduction in the contractile response as compared to maximal doses. Calculated on a molar basis at ED_{50} levels, DADL was 4 times as potent as N-allylnormetazocine.

Table 1 shows the sphincter pressures, before and after treatment with maximally effective doses of different opioids. The changes in sphincter pressure with all these opioids were statistically significant ($P < .05$). The absolute and percentage changes in the sphincter pressure are also shown in this table.

The onset of the responses to all these agents when used in maximally effective doses was 5 to 10 sec after administration, and their effect lasted 3 to 5 min. Lower doses were associated with longer onset and shorter duration of action.

Influence of naloxone, adrenergic and cholinergic antagonists and tetrodotoxin on the effect of opioids which

TABLE 1

Effect of maximally effective doses of different opioids on LESP

Agonist	No. of Observations	LESP		Change	
		Before treatment	After treatment		
		mm Hg		mm Hg	%
Buprenorphine (0.25 μg/kg)	4	50.0 ± 2.8[a]	13.2 ± 1.2*	36.8 ± 3.1[b]	73.4 ± 3.1[b]
Ketocyclazocine (16 μg/kg)	6	45.5 ± 3.2	21.0 ± 4.0*	24.5 ± 2.2[b]	54.7 ± 6.9[b]
Meperidine (40 μg/kg)	6	47.2 ± 8.3	17.2 ± 6.1*	30.0 ± 3.6[b]	66.4 ± 6.3[b]
N-allylnormetazocine (8 μg/kg)	5	42.2 ± 5.6	97.0 ± 13.0*	54.6 ± 7.9[c]	129.5 ± 10.0[c]
DADL (2.5 μg/kg)	6	61.2 ± 12.8	128.7 ± 29.9*	67.7 ± 17.4[c]	107.0 ± 7.9[c]

[a] Values are mean ± S.E.
[b] Decrease in LESP.
[c] Increase in LESP.
* Significantly different from control ($P < .05$; paired t test).

TABLE 2

Influence of two doses of naloxone on the effect of opioid agonists (used in the maximallay effective dose) which cause inhibition in LESP

Agonist	No. of Obser-vations	Decrease in LESP					
		Control		Naloxone (400 μg/kg)		Naloxone (4 mg/kg)	
		mm Hg	%	mm Hg	%	mm Hg	%
Buprenorphine (0.25 μg/kg)	4	34.7 ± 3.3[a]	73.4 ± 3.1	9.2 ± 2.3*	21.5 ± 5.2*	8.7 ± 2.0*	15.6 ± 4.7*
Ketocyclazocine (16 μg/kg)	6	31.3 ± 4.7	55.6 ± 5.6	35.0 ± 7.6 (N.S.)	51.6 ± 11.0 (N.S.)	2.4 ± 1.2*	4.4 ± 2.6*
Meperidine (40 μg/kg)	6	42.2 ± 4.3	56.0 ± 3.9	31.0 ± 6.5 (N.S.)	40.0 ± 7.9 (N.S.)	13.2 ± 5.6*	21.5 ± 8.2*

[a] Values are mean ± S.E.
* Significantly different from control ($P < .05$; paired t test); N.S., not significantly different from control ($P > .05$; paired t test).

TABLE 3

Influence of antagonists on the effect of the opioid agonists which cause inhibition of LES

	No. of Obser-vations	Decrease in LESP					
		Buprenorphine (0.25 μg/kg)		Ketocyclazocine (16 μg/kg)		Meperidine (40 μg/kg)	
		mm Hg	%	mm Hg	%	mm Hg	%
Control	6	35.0 ± 3.4[a]	80.8 ± 4.6	24.5 ± 2.2	55.6 ± 5.6	36.0 ± 5.3	56.0 ± 3.9
Tetrodotoxin	6	35.8 ± 2.8 (N.S.)	82.6 ± 3.8 (N.S.)	27.2 ± 3.4 (N.S.)	54.7 ± 6.9 (N.S.)	12.7 ± 4.6*	18.8 ± 7.1*
Control	6	37.3 ± 4.6	80.8 ± 4.2	27.3 ± 4.4	60.6 ± 4.1	32.4 ± 3.4	60.2 ± 4.3
Propranolol	6	36.2 ± 4.8 (N.S.)	78.6 ± 5.9 (N.S.)	26.5 ± 4.3 (N.S.)	57.3 ± 4.4 (N.S.)	30.3 ± 4.8 (N.S.)	58.6 ± 6.2 (N.S.)
Haloperidol	6	37.8 ± 3.9 (N.S.)	81.3 ± 4.2 (N.S.)	28.3 ± 2.6 (N.S.)	63.0 ± 3.0 (N.S.)	28.4 ± 5.3 (N.S.)	54.8 ± 7.4 (N.S.)
Indometha-cin	6	39.4 ± 5.8 (N.S.)	83.2 ± 5.2 (N.S.)	29.0 ± 3.5 (N.S.)	63.7 ± 3.6 (N.S.)	32.8 ± 3.9 (N.S.)	61.3 ± 3.8 (N.S.)

[a] Values are mean ± S.E.
* Significantly different from control ($P < .05$; paired t test); N.S., not significantly different from control ($P > .05$; paired t test).

cause inhibition of LES. Table 2 summarizes the influence of two doses of naloxone, 400 μg/kg and 4 mg/kg, on the effect of opioid agonists in the doses that produced maximal effects. The smaller dose of naloxone effectively antagonized the effect of buprenorphine, but not of ketocyclazocine or meperidine.

Propranolol, haloperidol and indomethacin failed to modify the inhibitory responses of buprenorphine, ketocyclazocine and meperidine on LESP ($P > .05$; table 3).

Propranolol and haloperidol were chosen because their respective agonists have been shown to cause inhibition of the sphincter. Indomethacin, an inhibitor of prostaglandin synthesis, was used because enhanced synthesis of prostaglandins causes a fall in LES pressure (Rattan and Goyal, 1980).

The inhibitory response of meperidine was not modified by a combination of hexamethonium and atropine. In controls, the fall in LESP with meperidine was 80.5 ± 2.8, and in the presence of hexamethonium and atropine it was 85.0 ± 4.3% ($P > .05$; $n = 4$). Tetrodotoxin failed to modify the inhibitory response of buprenorphine and ketocyclazocine ($P > .05$), but it significantly antagonized the fall in LESP with meperidine ($P < .05$; $n = 6$; table 3).

Influence of tachyphylaxis on the effect of opioids

which cause inhibition of the LES. Table 4 summarizes the influence of tachyphylaxis to different opioid agonists on the effect of three opioids which cause inhibition of LESP. Tachyphylaxis to different agonists was easily produced, and no cross-tachyphylaxis was observed among buprenorphine, ketocyclazocine and meperidine, when examined at their maximally effective doses.

Influence of naloxone, phentolamine, methysergide, pyrilamine, atropine and tetrodotoxin on the effect of opioids which cause increase in LESP. As shown in table 5, naloxone in a dose of 400 μg/kg produced insignificant antagonism of both N-allylnormetazocine and DADL ($P > .05$). However, naloxone in the dose of 4 mg/kg produced significant antagonism of both N-allylnormetazocine and DADL ($P < .05$).

Phentolamine, methysergide, pyrilamine and atropine were chosen because their respective agonists cause contraction of the LES. Phentolamine, methysergide and pyrilamine failed to modify the rise in LESP caused by N-allylnormetazocine or DADL. Atropine, however, significantly antagonized ($P < .05$) the rise in LESP with N-allylnormetazocine, but it did not modify the excitatory effect of DADL ($P > .05$; $n = 6$; table 6).

Tetrodotoxin did not modify the effect of DADL, but it significantly antagonized the effect of N-allylnormetazocine ($P < .05$; $n = 6$; table 6).

Influence of DADL tachyphylaxis on the effect of DADL, N-allylnormetazocine, buprenorphine and keto-

TABLE 4

Lack of cross-tachyphylaxis among opioids which cause inhibition of LES

	No. of Observations	Decrease in LESP					
		Meperidine (40 μg/kg)		Buprenorphine (0.25 μg/kg)		Ketocyclazocine (16 μg/kg)	
		mm Hg	%	mm Hg	%	mm Hg	%
Control	6	30.6 ± 5.2[a]	53.9 ± 5.4	45.0 ± 10.0	75.1 ± 4.4	33.8 ± 8.2	54.2 ± 5.4
Meperidine-tachyphylaxis (4 mg/kg)	6	9.4 ± 1.9*	12.2 ± 11.4*	35.0 ± 6.1 (N.S.)	80.0 ± 5.0 (N.S.)	28.3 ± 6.6 (N.S.)	60.2 ± 4.0 (N.S.)
Control	6	45.3 ± 10.5	67.0 ± 7.1	40.3 ± 8.9	86.3 ± 5.4	37.5 ± 6.5	67.1 ± 9.5
Buprenorphine-tachyphylaxis (25 μg/kg)	6	36.2 ± 4.8 (N.S.)	54.6 ± 3.3 (N.S.)	13.3 ± 3.0*	29.8 ± 6.8*	27.8 ± 6.9 (N.S.)	63.9 ± 8.1 (N.S.)
Control	3	20.7 ± 4.0	62.2 ± 8.9	31.0 ± 6.8	82.5 ± 7.0	23.7 ± 3.5	61.9 ± 1.7
Ketocyclazocinetachyphylaxis (2 mg/kg)	3	19.0 ± 2.6 (N.S.)	71.6 ± 1.9 (N.S.)	33.7 ± 3.3 (N.S.)	77.0 ± 7.1 (N.S.)	5.7 ± 1.5*	13.3 ± 6.9*

[a] Values are mean ± S.E.

* Significantly different from control ($P < .05$; paired t test); N.S., not significantly different from control ($P > .05$; paired t test).

TABLE 5

Influence of two doses of naloxone on the effect of opioid agonists (used in the maximally effective dose) which cause contraction of LES

Agonist	No. of Observations	Increase in LESP					
		Control		Naloxone (400 μg/kg)		Naloxone (4 mg/kg)	
		mm Hg	%	mm Hg	%	mm Hg	%
N-allylnormetazocine (8 μg/kg)	5	53.8 ± 5.3[a]	117.0 ± 6.3	54.0 ± 4.5 (N.S.)	115.0 ± 10.6 (N.S.)	9.4 ± 2.2*	19.3 ± 4.1*
DADL (2.5 μg/kg)	6	81.5 ± 14.2	138.8 ± 12.0	67.5 ± 6.1 (N.S.)	131.9 ± 9.0 (N.S.)	10.2 ± 2.7*	12.2 ± 7.3*

[a] Values are mean ± S.E.

* Significantly different from control ($P < .05$; paired t test); N.S., not significantly different from control ($P > .05$; paired t test).

TABLE 6

Influence of antagonists on the effect of the opioid agonists which cause contraction of LES

	No. of Observations	Increase in LESP			
		N-allylnormetazocine (8 μg/kg)		DADL (2.5 μg/kg)	
		mm Hg	%	mm Hg	%
Control	6	63.2 ± 6.9*	117.0 ± 6.3	62.3 ± 5.2	139.5 ± 14.3
Tetrodotoxin	6	13.8 ± 1.3*	25.0 ± 2.2*	61.2 ± 4.8 (N.S.)	153.2 ± 12.8 (N.S.)
Control	6	50.2 ± 8.7	112.6 ± 13.8	65.0 ± 6.2	143.4 ± 6.3
Atropine	6	11.4 ± 3.6*	38.2 ± 2.1*	60.6 ± 4.0 (N.S.)	141.6 ± 4.1 (N.S.)
Control	6	49.4 ± 13.5	122.7 ± 23.8	60.2 ± 6.2	135.3 ± 5.8
Phentolamine	6	62.5 ± 11.3 (N.S.)	129.4 ± 14.7 (N.S.)	63.3 ± 6.8 (N.S.)	144.8 ± 2.9 (N.S.)
Methysergide	6	49.5 ± 19.7 (N.S.)	141.3 ± 11.5 (N.S.)	68.3 ± 10.3 (N.S.)	130.8 ± 10.8 (N.S.)
Pyrilamine	6	51.4 ± 3.6 (N.S.)	118.4 ± 15.8 (N.S.)	64.3 ± 6.7 (N.S.)	142.6 ± 9.9 (N.S.)

[a] Values are mean ± S.E.

* Significantly different from control ($P < .05$; paired t test); N.S., not significantly different from control ($P > .05$; paired t test).

TABLE 7
Lack of cross-tachyphylaxis among opioids which cause contraction of the LES

	No. of Observations	Increase in LESP			
		DADL (2.5 μg/kg)		N-allylnormetazocine (8 μg/kg)	
		mm Hg	%	*mm Hg*	%
Control	6	65.5 ± 13.6[a]	107.0 ± 5.2	83.8 ± 17.6	151.3 ± 20.6
DADL tachyphylaxis (2.5 μg/kg/min)	6	21.5 ± 9.3*	18.6 ± 3.4*	85.8 ± 15.8 (N.S.)	171.3 ± 31.8 (N.S.)

[a] Values are mean ± S.E.
* Significantly different from control ($P < .05$; paired *t* test); N.S., not significantly different from control ($P > .05$; paired *t* test).

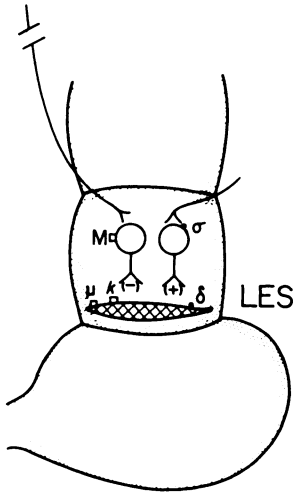

Fig. 4. Diagrammatic representation of localization of different opioid receptors on LES. *Mu* receptors (activated by buprenorphine) and *kappa* receptors (activated by ketocyclazocine) which mediate fall in LESP are present on the muscle. Meperidine (M) receptors lie on noncholinergic-nonadrenergic inhibitory neurons. On the other hand, *sigma* opioid receptors (activated by N-allylnormetazocine) that cause rise in LESP may lie on the cholinergic neurons and *delta* opioid receptors (activated by DADL) may lie on the LES muscle.

cyclazocine. Continuous i.a. infusion of DADL at the rate of 2.5 μg/kg/min significantly antagonized the response of an i.a. bolus of 2.5 μg/kg of DADL ($P < .05$; table 7).

In the presence of DADL tachyphylaxis, the effect of N-allylnormetazocine was not significantly antagonized ($P > .05$; table 7). The inhibitory effects of buprenorphine and ketocyclazocine on LES were also not modified by the presence of DADL tachyphylaxis ($P > .05$). The experiments using tachyphylaxis with N-allylnormetazocine were not performed due to a limited supply of the compound.

Effect of naloxone on LESP. Intravenous naloxone in the dose of 400 μg/kg had no significant effect on the resting sphincter pressure. The resting LESP at the time of naloxone administration was 68.0 ± 5.6 mm Hg, and 10 min later it was 69.7 ± 7.7 mm Hg ($P > .05$). However, 4 mg/kg of naloxone caused an immediate and transient fall in LESP which recovered within 2 min. The LESP values 10 min after 4 mg/kg were not significantly different from preinjection levels. The resting LESP at the time of naloxone (4 mg/kg) administration was 62.6 ± 7.9 mm Hg, and 10 min after naloxone, the LESP was 60.2 ± 4.8 mm Hg ($P > .05$; $n = 5$).

Discussion

The present studies suggest the existence of five distinct types of opioid receptors in the opossum lower esophageal sphincter as shown in figure 4. These receptor types are distinguished on the basis of differences in the effects of agonists, lack of cross-tachyphylaxis, differences in sensitivity to antagonism by naloxone and location on the sphincter muscle, noncholinergic, nonadrenergic inhibitory neurons or cholinergic excitatory neurons.

Activation of *mu*, *kappa* and meperidine receptors causes inhibition of the LES tone. *Mu* and *kappa* receptor activation is known to cause inhibition of electrically stimulated guinea-pig ileum (Lord *et al.*, 1977; Su *et al.*, 1981; Yoshimura *et al.*, 1982). In this preparation *mu* and *kappa* receptors are thought to be located on the cholinergic neurons, where they act to inhibit the release of acetylcholine (Paton, 1957; Karas and North, 1981). In contrast, the *mu* and *kappa* receptors of the LES are present directly on the sphincter muscle. This conclusion is based upon the observation that the inhibitory effect of *mu* and *kappa* receptor activation is not modified by tetrodotoxin, which antagonizes neural activity, and antagonists of neurohumoral substances which are known to cause inhibition of the LES. The *mu* and *kappa* receptors are separate because of lack of cross-tachyphylaxis among their agonists. Activation of *mu* receptors is far more sensitive to antagonism by naloxone than is activation of *kappa* receptors.

In contrast to the inhibitory effect of *mu* receptor activation on the LES, morphine (a *mu* receptor agonist) causes contraction of the ileocecal sphincter (Ouyang *et al.*, 1982) and ileum in several mammalian species (Pruitt *et al.*, 1974). The excitatory effect of morphine in canine ileum is mediated *via* the release of 5-hydroxytryptamine (Burks, 1973).

The most interesting finding of our study was that meperidine also caused inhibition of the LES, but this action was not mediated *via* the known opioid receptors. Meperidine appeared to activate another unidentified opioid receptor. The meperidine receptor does not show cross-tachyphylaxis to *mu* or *kappa* receptor agonists. Its sensitivity to naloxone is intermediate between *mu* and *kappa* receptors. Unlike the *mu* and *kappa* receptors, meperidine receptors appear to be present on the noncholinergic, nonadrenergic inhibitory neurons. This conclusion is based upon the fact that the inhibitory effect of meperidine was antagonized by tetrodotoxin but not by antagonists such as atropine, propranolol, haloperidol, pyrilamine or indomethacin.

Activation of preganglionic cholinergic neurons which synapse with the inhibitory neurons could also explain the observed effect of meperidine. However, this possibility was excluded as ganglionic block with the combination of atropine and hexamethonium failed to modify the inhibitory effect of meperidine.

Meperidine is an opioid used commonly in clinical practice. It causes inhibition of LES in man (Hey *et al.*, 1981) and is generally considered to have fewer spasmogenic effects than morphine on the gut (Jaffe and Martin, 1980). Noncholinergic,

nonadrenergic neurons are widely distributed in the gut and other organs (Gershon and Erde, 1981). It is possible that meperidine may act by activating such neurons in other parts of the gut as well. Commonly used antidiarrheal agents such as diphenoxylate and loperamide may resemble meperidine rather than morphine (Jaffe and Martin, 1980). It would be interesting to investigate the receptor activated by these agents.

Activation of *sigma* and *delta* receptors on the LES causes contraction of the sphincter. The *sigma* receptor is localized on the cholinergic excitatory neurons whereas *delta* opioid receptors are present on the sphincter muscle. The excitatory effects of N-allylnormetazocine are antagonized by atropine and tetrodotoxin whereas those of DADL are not. In the guinea-pig ileum *sigma* and *delta* receptors may be present but their activation causes inhibition of electrically stimulated contractions (Su *et al.*, 1981; Egan and North, 1981). *Delta* receptor activation causes contraction of feline ileum and ileocecal sphincter (Ouyang *et al.*, 1982). The excitatory effect of DADL on the feline ileum and ileocecal sphincter is exerted directly on the sphincter muscle (Ouyang *et al.*, 1982).

Recent demonstration of endogenous opioid-containing neurons in the myenteric plexus (Linnoila *et al.*, 1978; Schultzberg *et al.*, 1978; Uddman *et al.*, 1980) suggests that opioid may be involved in the physiological regulation of gastrointestinal functions. Our studies did not explore the role of opioid receptors in the physiological regulation of the LES function. However, they identify and localize five distinct opioid receptors in the LES and provide a basis for the effects of endogenous and exogenous opioid on the lower esophageal identification of different opioid receptors in a single organ.

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