verification of lesion sites at the end of testing revealed tissue damage 1.2 to 1.5 mm in diameter restricted to both raphe nuclei and the tissue rostral to them. These lesions produced anorexia and hyperdipsia in the first postoperative week, as previously reported (*7*). By the time of MH surgery, food intake, water intake, and weight gain were normal.

9. With heads flat between lambda and bregma skull sutures, 55°C heat lesions (*8*) were produced in each hemisphere for 1 minute at AP, +5.5 mm; ML, 0.5 mm; and DV, −8.6 to −9.0 mm. Lesions were 2.5 mm in diameter and 3.0 mm in length, extending from the anterior hypothalamus to the posterior ventromedial nuclei. Three rats with sham + MH lesions ate so voraciously that they suffocated within 48 hours of surgery; one sham + sham and one other sham + MH rat died from congestion during anesthesia.
10. All statistical comparisons were based on *t*-tests or Pearson product moment correlations. Probabilities reported are two-tailed unless predicted by previous results and indicated as one-tailed.
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14. Several other behavioral tests made on all rats will be reported elsewhere (D. V. Coscina, R. A. McArthur, H. C. Stancer, in preparation). Forebrain tissue was obtained, prepared, and analyzed by fluorometric assays as described before (*5*).
15. Radioenzymatic assays (D. V. Coscina and K. Lloyd, in preparation) of choline acetyltransferase and glutamic acid decarboxylase activities in seven brain regions from eight other rats with raphe lesions and six normal controls revealed no substantial differences. These data further support the specificity of our raphe lesions to forebrain 5-HT neurons.
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17. We recently found that 10 days after dorsal and median raphe lesions, 5-HT turnover following injection of ^{14}C-labeled 5-hydroxytryptophan is increased [J. J. Warsh, D. V. Coscina, H. C. Stancer, *Brain Res. Bull.* **1**, 273 (1976)]. Conceivably, the additional destruction of 5-HT neurons after MH lesions (*6*) would require some time to permit similar compensatory changes in the face of existing metabolic demands on residual neurons. At the same time, the sensitivity of postsynaptic 5-HT receptors might change with time [see Y. Agid, F. Javoy, J. Glowinski, *Nature (London)* **245**, 150 (1973) for a similar suggestion concerning less than totally destroyed DA neurons]. To add to these complexities, NE neurons damaged by MH lesions (*4–6*) could show similar time-dependent changes. Such changes, acting alone or in concert with 5-HT changes, might eventually permit reduced MH hyperphagia and weight gain.
18. Even after substantial weight loss, once-fat individuals display aberrant food preferences characteristic of the obese (*2*, p. 83).

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Endogenous Opiate Receptor Ligand: Electrically Induced Release in the Guinea Pig Ileum

Abstract. *Opiate receptors mediate the electrically evoked inhibition of the myenteric plexus–longitudinal muscle preparation of the guinea pig ileum. The electrically induced activation of the opiate receptor was produced by a prolonged simulation at 10 hertz and provides the first evidence that an endogenous opiate receptor ligand is released by nerve stimulation. The specificity of the phenomenon was demonstrated by the reversal obtained with the narcotic antagonists naloxone, naltrexone, and GPA 1843; GPA 1847, the (+)-isomer of 1843, did not cause reversal. The model system described should be useful for the study of the storage, synthesis, and release of endorphins.*

Although endorphins (*1*), which are endogenous opiate-like peptides (*2, 3*), and opiate receptors (*4, 5*) are found in the central and peripheral nervous systems, the effects of opiate antagonists described so far are surprisingly few and of small magnitude. It has been reported that the reaction time to nociceptive stimuli can be reduced by the narcotic antagonist naloxone (*6*). These observations suggest that endorphins may have an inhibitory effect on the neuronal pathways that mediate nociceptive effects. The effects of narcotic antagonists have not yet been demonstrated on the electrically induced contractions of the myenteric plexus–longitudinal muscle preparation of the guinea pig ileum; however, this tissue is known to be exquisitely sensitive to opiates (*7, 8*), to bind stereospecifically labeled narcotic agonists and antagonists (*4, 8*), and to contain enkephalin (*3*). The only effect that opiate antagonists have on this preparation has been described by Waterfield and Kosterlitz (*9*), who have demonstrated that naloxone produces a small increase in the output of acetylcholine evoked by field stimulation.

Since the effects of opiates and exogenous endorphins on the electrically stimulated guinea pig ileum can only be demonstrated at very low frequencies of stimulation such as 0.1 to 0.017 hertz (*10*), most of the studies on the effects of naloxone on this system have been carried out at low frequencies. We thought that the optimal frequency for the release of endorphins might be higher than 0.1 hertz, and therefore that the peptide release might not be apparent when the frequency of stimulation is optimized to demonstrate its opiate-like effects.

In attempts to provide evidence for the release of endorphins from the guinea pig ileum, we combined periods of stimulation at different frequencies. Initial experiments demonstrated that a naloxone-sensitive inhibition of the contractions of the longitudinal muscle could be elicited after periods of stimulation at 10 hertz.

The myenteric plexus–longitudinal muscle strip was prepared as described by Paton and Zar (*11*) and Kosterlitz *et al.* (*12*). Each strip was suspended in a 10-ml organ bath containing Krebs-bicarbonate solution at 37°C and gassed with a mixture of 95 percent O_2 and 5 percent CO_2; the composition of the Krebs solution differed from that previously described in that it contained $3 \times 10^{-5} M$ choline chloride (*13*). The isometric contractions of the muscle were registered with a Grass force transducer (model FT 03C) coupled to a Grass polygraph; the tension of the strip was maintained at 0.3 g. The tissue was stimulated through two platinum ring electrodes with supramaximal rectangular pulses of 1-msec duration applied at a frequency of 0.1 hertz. The opiate-like inhibition was elicited by a 5-minute stimulation of the same voltage and pulse duration but at a frequency of 10 hertz. The electronic equipment used has been described (*13*).

A marked inhibition of the basal contractions appears after the stimulation at 10 hertz (Fig. 1, top). The degree of inhibition is a function of the duration of the period of stimulation as well as frequency, voltage, and duration of the puls-

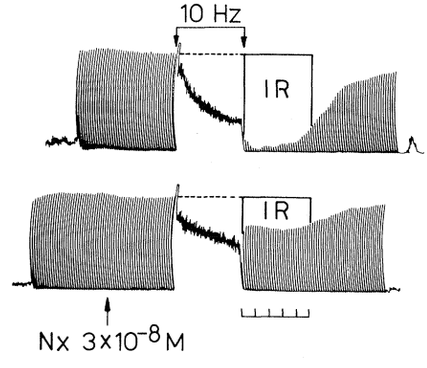

Fig. 1. Inhibitory response (IR) of the myenteric plexus–longitudinal muscle preparation elicited by stimulation at 10 hertz (top) and reversal by naloxone (bottom). The area of the recorded contractions generated during 5 minutes by stimulation at 0.1 hertz was measured before (basal response, BR) and immediately after stimulation at 10 hertz (post-stimulation response, PSR). The inhibitory response was calculated by subtracting the PSR from the BR. Naloxone (Nx), at the concentration indicated, was added 5 minutes before the stimulation at 10 hertz in order to measure the BR in the presence of the drug (the horizontal calibration is 1 minute per division).

es (*14*). The electrically induced inhibition is substantially reversed by a small concentration of naloxone (Fig. 1, bottom). Naltrexone, also a potent narcotic antagonist, can also reverse the electrically evoked inhibition. The dose-response curves for the reversal produced by naloxone and naltrexone are shown in Fig. 2. The EC_{50} (the concentration required to produce a 50 percent effect) is 10 n*M* for naltrexone and 25 n*M* for naloxone. On this basis, naltrexone is 2.5 times more potent than naloxone; it is noteworthy that naltrexone is three times more potent than naloxone in antagonizing normorphine in the same preparation (*15*).

To rule out the possibility that the antagonism described here might be due to a nonspecific effect rather than to the interaction of the narcotic antagonists with the opiate receptor, we tested a pair of opiate antagonists having a different structure. These antagonists are the two optical isomers of *N*-allyl-β-9-methyl-5-phenyl-2′-hydroxy-6,7-benzomorphan; the (−)-isomer (called GPA 1843) is about 60 times more potent than the (+)-isomer (GPA 1847) when tested in the same preparation against normorphine (*9*). We found that GPA 1843 reversed the electrically induced inhibitory response with an EC_{50} of $1.5 \times 10^{-7}M$ (Fig. 2). In contrast, the (+)-isomer required high concentrations to produce any measurable reversal. These high concentrations induced nonspecific effects that precluded our obtaining a reliable dose-response.

The opiate-like nature of the electrically evoked inhibition is further supported by the finding that small concentrations of morphine ($1 \times 10^{-8}M$ to $3 \times 10^{-8}M$) markedly potentiate the inhibition while dextrorphan is inactive at similar concentrations.

The opiate antagonists reversed about 70 to 75 percent of the electrically evoked inhibition of the myenteric plexus–longitudinal muscle preparation. This is remarkably high considering that this preparation contains adrenergic (*16*) and purinergic (*17*) inhibitory nerves that most likely contribute to the observed inhibition.

The following characteristics of the electrically evoked inhibition demonstrate that it is due to the activation of the guinea pig ileum myenteric plexus opiate receptors.

1) The inhibition is reversed by the potent narcotic antagonists naloxone and naltrexone, each at an EC_{50} that is well within the concentration at which these drugs are known to exert their specific effects.

2) The reversal is independent of the general structure of the antagonists: naloxone and naltrexone are oxymorphone derivatives while the GPA compounds are benzomorphan derivatives.

3) The reversal is stereospecific as demonstrated by the higher potency of GPA 1843, the (−)-isomer, when compared to GPA 1847, the (+)-isomer.

4) The order of potency of the antagonists tested (naltrexone > naloxone > GPA 1843 > GPA 1847) on the reversal of the electrically produced inhibition is the same as the order of potency of the same compounds to reverse the inhibitory effects of opiates on the same preparation.

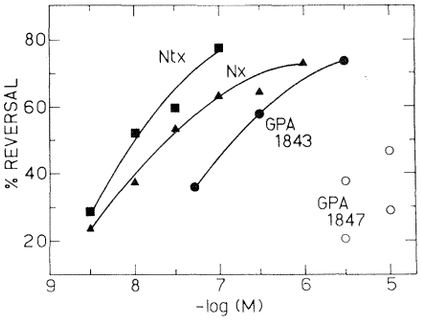

Fig. 2. Dose-response curves of the reversal of the opiate-like inhibition of the myenteric plexus–longitudinal muscle preparation. The percent reversal (ordinate) was determined as follows. The area of the recorded contractions, generated during 5 minutes by stimulation at 0.1 hertz, was measured before (basal response, BR) and immediately after stimulation at 10 hertz (poststimulation response, PSR). The percent inhibitory response (IR) was calculated as (BR − PSR/BR) × 100. The antagonism of the inhibitory response, represented as the percentage reversal, was calculated as follows:

$$\%\ \text{reversal} = \frac{\%IR_c - \%IR_e}{\%IR_c} \times 100$$

where IR_c is the inhibitory response of the control and IR_e is the inhibitory response in the presence of the drug. The abscissa is the negative logarithm of the drug concentration (*M*). Drugs were added 5 minutes before the stimulation at 10 hertz in 30 or 40 μl of water. The preparation was washed four to six times during the 45-minute rest period that followed each determination; Ntx, naltrexone (squares); Nx, naloxone (triangles); GPA 1843 (closed circles); and GPA 1847 (open circles). The dose-response curves are the average of at least three complete sets of determinations. The dose-response for GPA 1843 was obtained with three points that bracket the EC_{50} because the long duration of action of this antagonist (*15*) limits the number of points obtainable from a single strip. The GPA 1847 experiment was repeated four times but most points had to be discarded due to nonspecific drug effects.

The activation of the opiate receptors of the myenteric plexus elicited by electrical stimulation suggests that the phenomenon described is due to the release of endorphins. The model system described will be very useful for studying the synthesis of endorphins and the mechanisms of its release by nerve stimulation as well as the effects of drugs that might affect these processes.

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