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CALCIUM REQUIREMENTS FOR ELECTRICALLY-INDUCED RELEASE OF AN ENDOGENOUS OPIATE RECEPTOR LIGAND FROM THE GUINEA-PIG ILEUM

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Calcium requirements for electrically-induced release of an endogenous opiate receptor ligand in the myenteric plexus-longitudinal muscle strip of the guinea-pig ileum were studied. The naloxone-reversible depression of the electrically evoked contraction caused by stimulation at 10 Hz in normal Krebs solution was markedly reduced by decreasing the calcium concentration in the solution. The depression was greatly diminished by increasing the magnesium concentration in the solution. These results show that the electrically-induced release of an opiate-like material requires calcium ions.

Introduction Recent studies on the distribution of an opiate-like material such as methionine- or leucine-enkephalin suggest that an opioid material may act as a neurotransmitter or neuromodulator associated with opiate receptors in the central and peripheral nervous system (Elde, Hökfelt, Johansson & Terenius, 1976; Simantov, Kuhar, Uhl & Snyder, 1977; Hökfelt, Ljungdahl, Terenius, Elde & Nilsson, 1977). It has been reported that an endogenous opiate receptor ligand in the myenteric plexus-longitudinal muscle strip of the guinea-pig ileum can be released by electrical field stimulation at 10 Hz (Puig, Gascón, Craviso & Musacchio, 1977). In the present investigation, we studied the calcium requirements for the electrically-induced release of an endogenous opiate receptor ligand, a possible neurotransmitter in the strip (Hughes, Kosterlitz & Smith, 1977), since the release of established neurotransmitters such as acetylcholine and noradrenaline requires calcium ions (see review by Rubin, 1970).

Methods The myenteric plexus-longitudinal muscle strip of the guinea-pig ileum was prepared as described by Paton & Zar (1968). The strip was suspended in a 6 ml organ bath containing Krebs-bicarbonate solution of the following composition (mM): NaCl 118, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 1.19, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11 and choline chloride 0.02 at 37°C and bubbled with 95% O_2 and 5% CO_2; stimulation was through two platinum ring electrodes with supramaximal rectangular pulses of 1 ms duration. Low-calcium or high-magnesium solution contained the calcium or magnesium concentration described in the Results section and the other components as described above. Calcium-free solution contained 4.8 mM $MgSO_4$ and 2.4 mM 1,2,*bis*,2 amino ethoxyethane-NNN'N'-tetra-acetic acid (EGTA) and the other components as described above. Contractions of the strip were detected by means of a force-displacement transducer with the resting tension adjusted to 0.5 g and displayed on ink-writing polygraphs. The basal contractions of the strip were elicited by stimulation at 0.1 Hz. The release of an opiate-like material was elicited by periods of stimulation of the same voltage and pulse duration but at a frequency of 10 Hz. The area of the recorded contractions generated during 5 min by stimulation at 0.1 Hz was measured before (basal response, BR) and immediately after stimulation at 10 Hz (post-stimulation response, PSR) as reported by Puig *et al.* (1977). The inhibitory response was calculated by subtraction of the PSR from the BR. The percentage inhibitory response was calculated as (BR − PSR/BR) × 100.

Results The basal contractions of the myenteric plexus-longitudinal muscle strip, which were elicited by stimulation at 0.1 Hz, were markedly inhibited after stimulation for 3 min at 10 Hz. The inhibition was reversed by naloxone (10^{-6} M), a finding consistent with that obtained by Puig *et al.* (1977). Effects of calcium ions on the opiate-like inhibition were studied by replacing normal Krebs solution by low-calcium or calcium-free solution 30 s before and during stimulation at 10 Hz. On exposure to low-calcium or calcium-free Krebs solution, the basal contractions of the strip were greatly reduced in amplitude or completely disappeared (Figure 1). When the strip was stimulated for 3 min at 10 Hz in these solutions, the inhibition was greatly decreased (Figure 1b and c) as compared with that in normal Krebs solution. The magnitude of the electrically-induced inhibition was dependent on calcium concentration in the solution;

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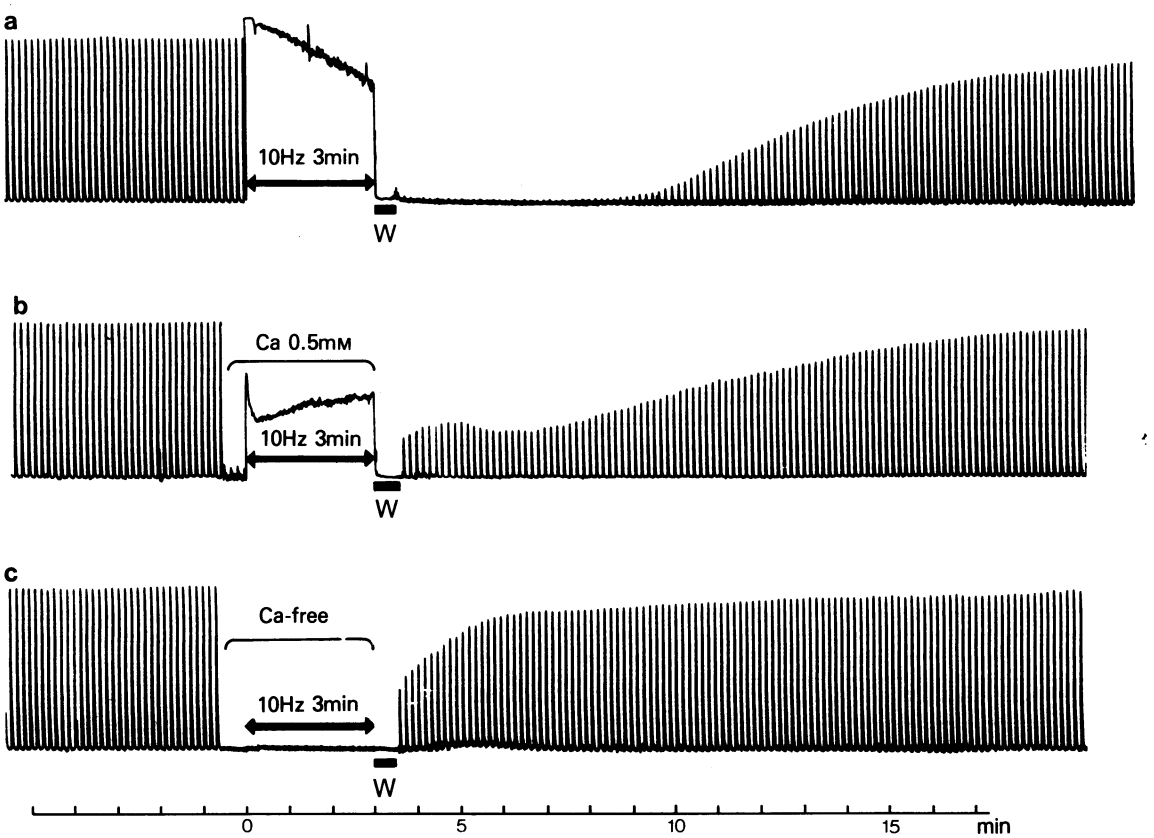

Figure 1 Inhibitory response of the myenteric plexus-longitudinal muscle preparation elicited by stimulation at 10 Hz in normal (a), low-calcium (b) and calcium-free (c) Krebs solutions. Normal Krebs solution was replaced by low-calcium or calcium-free solution 30 s before and during stimulation at 10 Hz. After stimulation for 3 min at 10 Hz, the strip was washed three times (indicated by W) with 6 ml normal Krebs solution. The strip was then stimulated at 0.1 Hz in normal Krebs solution.

i.e., 100% inhibition obtained with 2.54 mM, 92% with 0.76 mM, 69% with 0.50 mM, 24% with 0.25 mM $CaCl_2$ and 17% in calcium-free solution. The inhibition was also greatly diminished by increasing the concentration of magnesium in the solution; i.e., 88% with 6 mM $MgSO_4$ and 51% with 12 mM $MgSO_4$.

Discussion The results of the present investigation show that the electrically-induced release of an endogenous opiate receptor ligand in the guinea-pig ileum requires calcium ions, providing additional evidence that an opiate-like material may act as a neurotransmitter associated with opiate receptors.

The requirement of calcium ions for the release of an opioid material is suggested by the fact that the usual marked inhibitory effect after stimulation at 10 Hz, was greatly decreased in the low-calcium or calcium-free solution. In addition, the magnitude of the electrically-induced inhibition was dependent on calcium concentration in the solution; i.e., the lower the calcium concentration, the lower the magnitude of inhibition. Moreover, the electrically-induced inhibition was markedly diminished by increasing the concentration of magnesium ions in the solution; increase in magnesium ions antagonizes the actions of calcium ions in many biological systems (Rubin, 1970). While this manuscript was in preparation, it was reported that the potassium-induced release of enkephalins from brain slices and synaptosomes was calcium-dependent (Henderson, Hughes & Kosterlitz, 1978; Iversen, Iversen, Bloom, Vargo & Guillemin, 1978), observations which support the present results.

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