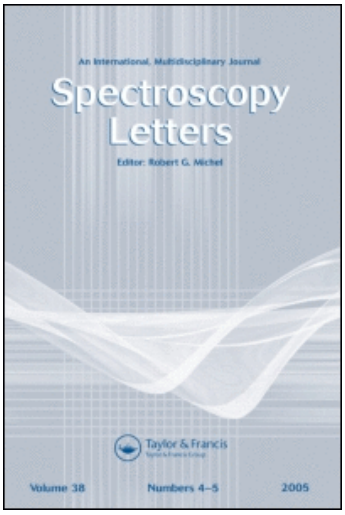

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A STUDY OF POSSIBLE MORPHINE COMPLEXES FORMED WITH CALCIUM AND MAGNESIUM

Keywords: Morphine, Ca^{++}, Mg^{++} Complexes, Fluorescence, Potentiometric Titration

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ABSTRACT

Morphine fluorescence spectra were obtained for morphine sulfate in distilled water, in KRP solution, and in KRP solutions with various concentrations of Ca^{++} and Mg^{++}. No evidence of fluorescence shifting indicative of complex formation was observed. Similar solutions were used in a potentiometric study, and no evidence for complex formation was observed.

INTRODUCTION

Calcium has been implicated in many functions involving the nervous system, particularly in release of neurotransmitters and neuronal excitability[1]. Rubin and Douglas[2] showed that acetylcholine secretion from the adrenal medulla was diminished in a

calcium-free Locke solution and enhanced in a calcium rich solution. Norepinephrine release *in vitro* from presynaptic nerve endings from rat brain has been demonstrated to depend upon calcium for release[3]. Calcium is involved in the post-synaptic actions of neurotransmitters[4], and in the propagation of nerve impulses[5]. Calcium also alters the permeability of nerve membranes[6], alters, *in vitro*, the activity of nerve cell activity[7] and is a primary transducer of many hormone receptor interactions[8].

Magnesium appears to compete with calcium for membrane binding sites[9] and interferes with Ca^{++} entry into presynaptic terminals[10]. The Mg^{++} ion is also thought to have an effect on the sodium pump and is a known cofactor in Na^{+} -K^{+} ATPase[11].

There have been many reports that calcium may have a direct role in the analgesia produced by narcotic drugs. Morphine was found to inhibit potassium ion-stimulated oxygen uptake only in mediums with little or no calcium[12]. Halliday and Elliott[13] showed that morphine can depress the QO^2 content of rat cerebral cortex slices in the presence of calcium ions. Nishigishi[14] reported morphine initially caused an increase in blood calcium and Weger and Amsler[15] reported that a high calcium diet delayed the development of tolerance. Chronic morphine treatment also produces a progressive calcinuresis in rats[16] and a decrease in brain calcium[17], which can be blocked by the narcotic antagonist, naloxone[18].

Kakunaga, *et al.*[19] demonstrated that intracisternal injections of calcium into mice antagonized the analgesic effects of morphine.

This effect was also demonstrated by Kaneto[20]. Harris, et al.[21] provided evidence that intracerebroventricular injection of Ca^{++}, Mg^{++} of Mn^{++} antagonized the analgesic effects of morphine but Sr^{++}, Ba^{++}, Ni^{++}, Hg^{++} and Cd^{++} were not antagonistic. This work also demonstrated that pretreatment with pargyline or 6-hydroxydopamine did not alter the effect produced by the various ions and that the calcium ionophore X537A potentiated the narcotic antagonistic effect of low doses of Ca^{++}. Decalcifying agents have a weak analgesic effect themselves and potentiate the analgesia produced by morphine

The direct involvement of Mg^{++} in morphine analgesia is less substantial. Vachon and Marchand[22] have demonstrated that an acute dose of morphine caused hypermagnesemia in rats. The effect was dose-dependent and antagonized by nalorphine. Chronic morphine treatment was associated with an increased magnesium excretion[24]. Fong, et al.[25] showed Mg^{++} concentration changes in skeletal muscle microsomal fractions but not in the nuclear or mitochondrial fraction of chronically morphinized rats. Devynclz, et al.[26] showed that the action of leval-lorphan upon cultures of E. colli is dependent upon the concentrations of Ca^{++} and Mg^{++} in the medium.

The importance of the Ca^{++} and Mg^{++} ions in analgesia has prompted this study of the possibility of complex formation with morphine and naloxone. Our preliminary semiempirical calculations indicated that such a complex is formed. A spectrofluorometric

study[21] had indicated the possibility of morphine-formed complexes with Ca^{++} and Mg^{++}. Our first effort then was directed toward attempting to repeat that spectrofluorometric study.

Methods

Modified Kreb's Ringer Phosphate (KRP) buffers with pH 7.4 ± 0.007 were prepared containing 6.5 mM NaCl, and the following mM ratios of $CaCl_2$ to $MgSO_4$; 0:1.3, 0.5:13, 1.0:1.3, 1.5:1.3, 2.0:1.3, 1.0:0.0, 1.0:0.65, 1.0:1.95, and 1.0:2.6. A reference buffer was also prepared containing no Ca^{++} of Mg^{++}. The KCl, NaCl, and $MgSO_4$ were obtained from the J. T. Baker Co. and were "Analyzed" grade reagents. The KH_2PO_4 and K_2HPO_4 were obtained from Matheston, Coleman and Bell were enzyme quality reagents.

Morphine sulphate, shown to be pure by thin layer chromatography, was incubated in the buffers at 37° C. The fluoresence spectra of the incubated solutions was measured in an Aminco - Bowman Spectrophotofluorometer obtained from the American Instrument Co. of Silver Spring, Maryland equipped with a Mercury-Zenon Arc Lamp and a 1 P21 slide on photomultiplier tube. Slit widths were 2 nm except for the pm slit width which was 5 nm.

In the potentiometric experiment, a Radiometer pH M 56 pH meter was used with a Gilmont microburette. The experiment was performed under a N_2 atmosphere and using a water-jacketed titration vessel kept at 25.0 ± 0.1 °C. The titrant was NaOH at 0.218 M/0.1 M ionic strength with $NaClO_4$. The morphine concentration was 3-4 x 10^{-3} M, the [Ca^{++}] was 5.27 x 10^{-3} M, and the [Mg^{++}] was 5.60 x 10^{-3} M.

RESULTS

Morphine sulphate (1 mM) was incubated in distilled H_2O for 30 minutes at 37°C and the fluorescence spectra determined (Figure 1). Excitation and emission maxima are seen at 290 and 360 nm

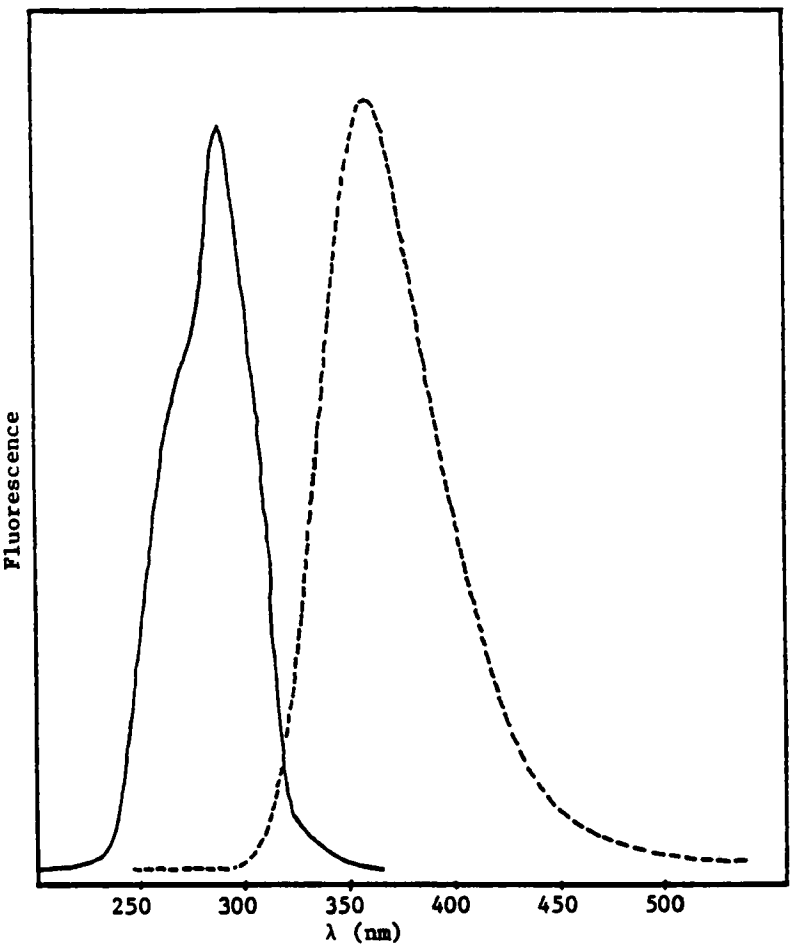

FIG. I

Fluorescence excitation (solid line) and emission (dotted line) spectra for morphine sulfate (1mM) in distilled water.

respectively. Fluorescence spectra were then obtained for the morphine sulphate (1 mM) incubated in KRP containing no Mg^{+2} or Ca^{+2} ions (Figure 2). No shift in the excitation/emission maxima of 290/360nm was indicated. Morphine sulfphate incubated in KRP containing 1.0 mM Ca^{+2} and 1.3 mM Mg^{+2} yields spectra which also

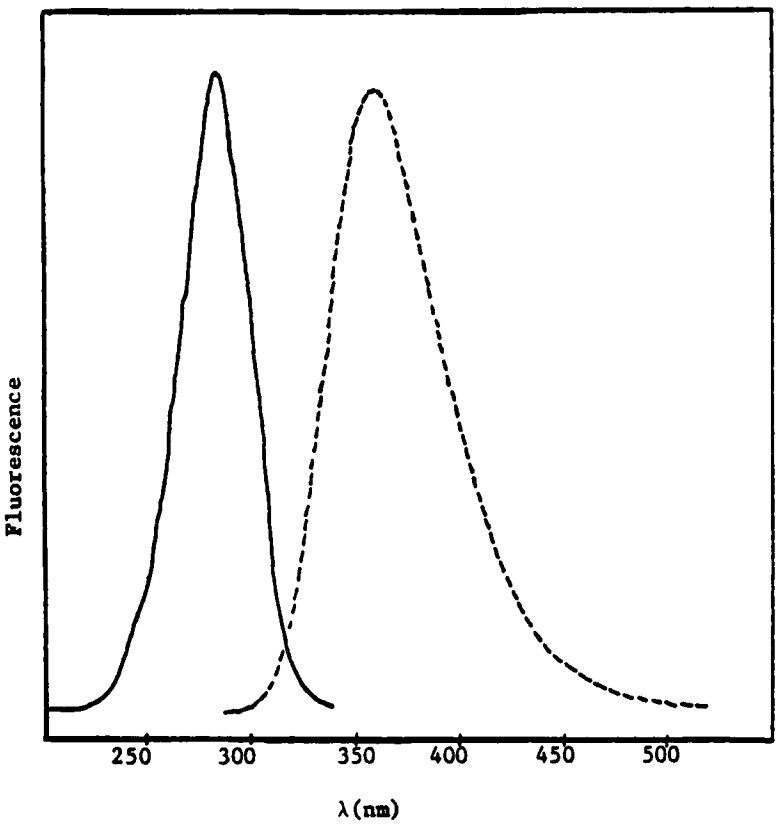

FIG. 2

Fluorescence excitation (solid line) and emission (dotted line) spectra for morphine sulphate (1mM) in KRP solution and no metal ions present

have excitation and emission maxima at 290 and 360 nm respectively. An example spectrum is shown in Figure 3. The lack of a shift of the maxima is taken as an indication that there is very little or no complexation of morphine sulphate with Ca^{+2} of Mg^{+2} ions.

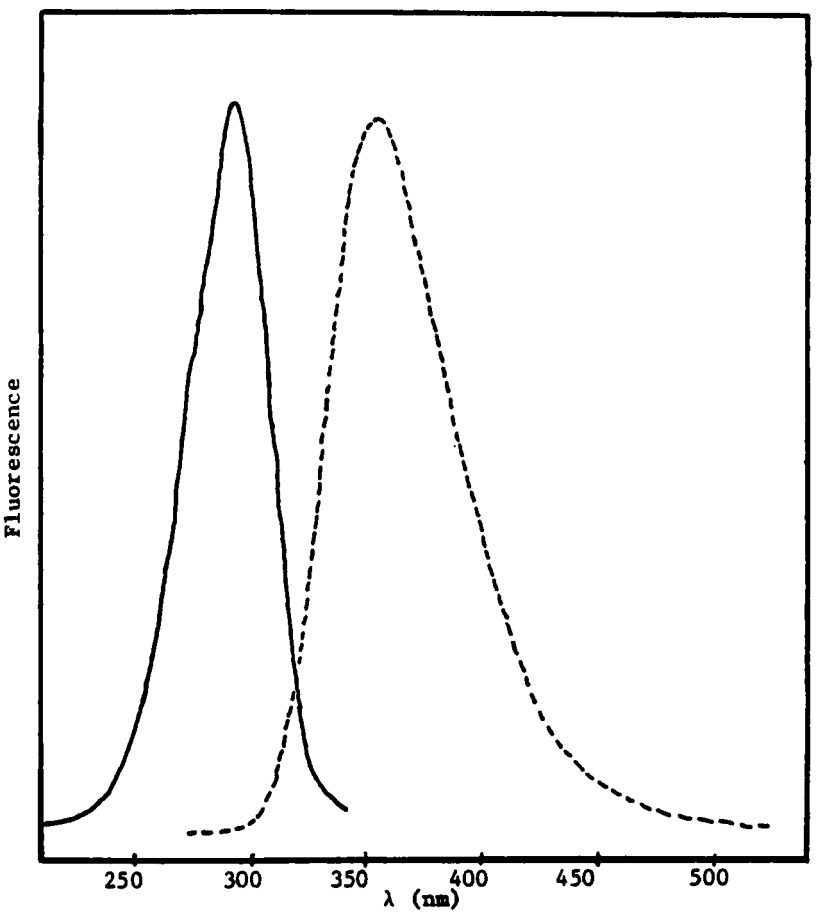

FIG. 3

Fluorescence excitation (solid line) and emission (dotted line) spectra for morphine sulphate (1mM) in KRP solution containing 1.0 mM Ca^{+2} and 1.3 mM Mg^{+2}.

In order to determine if either time or metal ion concentration would influence the formation of a complex, morphine sulphate (1 mM) was incubated at 37°C in KRP buffers pH 7.4 with the following Ca^{+2}/Mg^{+2} mM ratios: 0.0:0.0, 0.0:1.3, 0.5:1.3, 1.0:1.3, 1.5:1.3, 2.0:1.3, 1.0:0.0, 1.0:0.65, 1.0:1.95 and 1.0:2.6. Excitation and emission spectra were obtained for each solution immediately after preparation and then again after six hours of incubation. Excitation and emission maxima of all of the spectra collected were 305 and 360 nm. No shifting of the spectra to indicate complex formation was observed in any of the spectra.

Potentiometric titrations were performed on morphine solutions alone and in the presence of either Ca^{2+} or Mg^{2+}. Figure 4 shows the results of a typical titration. When titrated either alone or in the presence of metal ions, morphine precipitates at pH 8.2, redissolving again at pH 10.3. As is evident in Figure 4, the presence of Ca^{2+} has no detectable effect on the titration curve for morphine alone. Thus, if Ca^{2+} or mg^{2+} form complexes with morphine they must be very weak. A titration of morphine in the presence of 1.84×10^{-3} M Cu^{2+} also gave no detectable evidence for complex formation.

Based on these two sets of data, we must conclude that if morphine forms a complex with either calcium or magnesium, it is beyond the limits of detection of these experiments. This is in direct contrast with previously published data.[21] We would anticipate that there would thus be no complex formation of morphine with calcium or magnesium under physiological conditions.

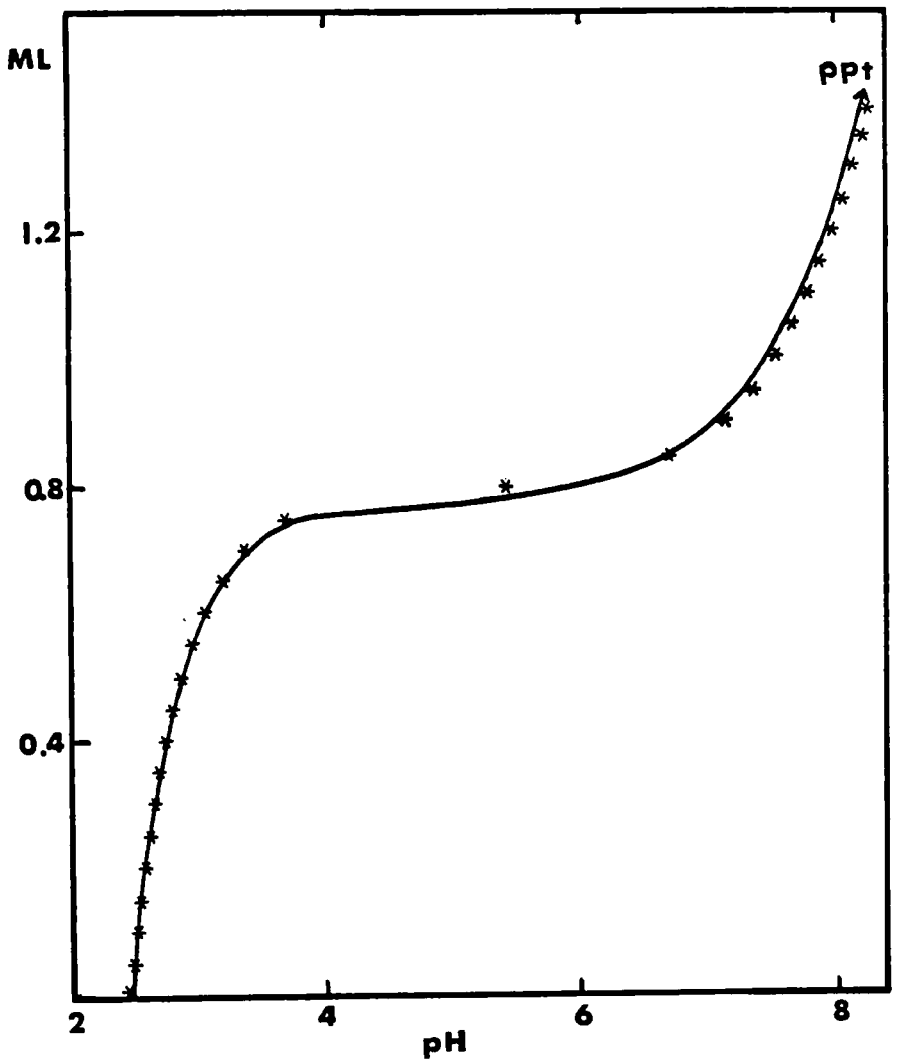

FIG. 4

Potentiometric titration of morphine with NaOH. The solid line is for titration of morphine alone ($[morphine]_t = 3.56 \times 10^{-3}$M). The starred points are for titration of morphine in the presence of Ca^{2+} ($[Ca^{2+}]_t = 5.27 \times 10^{-3}$M, $[morphine]_t = 3.54 \times 10^{-3}$M). Titrant is 0.218M NaOH, 25.0 ± 0.1°, $\mu = 0.1$ ($NaClO_4$).

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