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METAL ION INTERACTIONS WITH OPIATES

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INTRODUCTION

A number of monovalent and divalent metal ions are known to be vital in the normal functioning of the nervous sytem. However, investigations into the roles of inorganic ions in the actions of narcotic drugs have so far largely involved studies of Ca^{2+} effects, although experimental evidence has suggested that other ions such as Mg^{2+}, Mn^{2+}, Cu^{2+}, and Na^{+} may also play a part.

Opiate effects have often been associated with Ca^{2+}, which appears to have multiple functions in the nervous system. Thus calcium ions are a component of cell membranes, serving as a membrane stabilizer (1), they are involved in nerve impulse propagation (2, 3), and they alter nerve membrane permeability (4–6). Nerve stimulation causes an influx of extracellular calcium (7) while calcium ions are also necessary for stimulation-secretion coupling since neurotransmitter release is a calcium–dependent phenomenon (8–13). Furthermore, calcium ions are involved in postsynaptic neurotransmitter effects (14, 15) and in the reuptake of norepinephrine (16, 17).

In addition, calcium ions act as important regulators of a number of enzymes such as adenylate cyclase, guanylate cyclase, and phosphodiesterase (18–20). Calcium ions are also involved in protein phosphorylation by protein kinase (21, 22) and in the activities of tyrosine and tryptophan hydroxylases (23, 24).

The other metal ions that have so far been implicated in narcotic actions have less diverse roles in nervous system function. Changes in the disposition of sodium (as well as potassium) ions across nerve membranes results

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in the generation of the action potential, the central feature of nerve impulse production.

Magnesium and manganese ions can act as activators for a number of enzymes. Thus, for instance, both Mg^{2+} and Mn^{2+} (25) can increase adenylate cyclase activity. Copper is an essential factor in a number of oxidase enzymes such as cytochrome *c* oxidase (26) and is also a component of dopamine β-hydroxylase (27), an enzyme involved in the biosynthesis of norepinephrine. Cu^{2+}, Mg^{2+}, and Mn^{2+} may also be involved in monoamine binding, storage, and transport as a result of chelate formation between metal ion, neurotransmitter, and ATP (28).

In this article we attempt to present a comprehensive survey of research into metal ion involvement in narcotic actions. Kaneto (29) has produced such a review previously but in the ensuing period (during which time much relevant information has been collected) other review articles have either been very brief (30) or have concerned themselves with particular aspects of the research (31–33).

METAL ION EFFECTS ON ACUTE NARCOTIC ANALGESIA

Hano et al (34) reported that intracisternal injections of Ca^{2+} markedly antagonized the analgetic effects of morphine in mice. Potentiation of morphine analgesia was seen with intracisternal injections of ethylenediaminetetraacetic acid (EDTA), a chelator of Ca^{2+} and Mg^{2+}, while EDTA alone produced a weak analgesic effect which was diminished by equimolar doses of Ca^{2+}.

It was subsequently found (29, 35) that intracisternal injection of Ca^{2+} antagonized meperidine and ohton (dimethylthiambutene) as well as morphine analgesia. The chelating agents EDTA, cyclohexanediaminetetraacetic acid, sodium citrate, and sodium oxalate potentiated the analgesic effects of these drugs, while the Ca^{2+} complexes of the chelating agents, and also other cations alone (Mg^{2+}, Ba^{2+}, Sr^{2+}, Zn^{2+}, Fe^{2+}, Al^{3+}, K^{+}, and Na^{+}), had no effects on the analgesia produced by morphine. Ca^{2+} was again reported to antagonize morphine analgesia in mice (36) and pethidine analgesia in guinea pigs after subcutaneous (s.c.) administration of the ions (37).

Harris et al (38) also observed Ca^{2+} antagonism of morphine analgesia in both naive and morphine-tolerant mice after intracerebroventricular (i.c.v.) administration of the cations. In contrast to the earlier reports, however, Mg^{2+} (as well as Mn^{2+}) also antagonized morphine analgesia, while Sr^{2+}, Ba^{2+}, Ni^{2+}, Hg^{2+}, and Cd^{2+} had no effect. Again unlike the

earlier reports EDTA did not affect analgesia although another chelating agent, ethyleneglycol tetraacetic acid (EGTA), was an effective potentiator of morphine analgesia. The authors interpreted this difference as an indication of the importance of alterations in Ca^{2+} rather than Mg^{2+} in the actions of morphine, since EGTA (but not EDTA) has a much higher affinity for Ca^{2+} than Mg^{2+} (39). The ionophore X-537A greatly increased the morphine antagonist effects of a low dose of Ca^{2+}. Since X-537A increases the permeability of membranes to divalent cations (40) this would suggest that Ca^{2+} antagonizes morphine effects mostly at an intracellular site or sites. Treatment with pargyline (which elevates brain monoamine levels) or 6-hydroxydopamine (which depletes brain catecholamines) did not alter the morphine antagonist effect of Ca^{2+}, perhaps indicating that altered release of catecholamines is not involved in this action of Ca^{2+}. The apparent affinity of naloxone for its receptors, as measured by in vivo pA_2 determinations, was not altered by Ca^{2+} in either nontolerant or tolerant animals.

Because a relationship between Ca^{2+} and narcotic actions had been established, further investigations were made (41–43) in order to examine the possible analgesic properties of lanthanum (La^{3+}) and cerium (Ce^{3+}), agents which inhibit Ca^{2+} binding and movement across biological membranes (44). Intracerebral injections of La^{3+} in mice and rats and Ce^{3+} in mice produced analgesia, while a low dose of La^{3+} which itself had little analgesic effect significantly increased the analgesic effect of morphine. Furthermore, the effects of La^{3+} were antagonized by Ca^{2+} and also by naloxone, suggesting that the narcotic antagonist properties of naloxone may involve increased binding or transport of Ca^{2+}. Alternatively this may indicate that La^{3+} acts by releasing endogenous opiates and that it is the effect of the opiates which is reversed by naloxone. Such an explanation, however, needs to be reconciled with the fact that Ca^{2+} appears necessary for the release of endogenous opiate peptides (45).

Other similarities between La^{3+} and opiates were also demonstrated. Animals tolerant to morphine were also tolerant to La^{3+} although to a lesser extent. Moreover, La^{3+} reduced both abrupt and naloxone-precipitated withdrawal jumping in morphine-dependent mice. From experiments involving injections of morphine or La^{3+} into eight subcortical rat brain sites it was reported that these agents appeared to share the same sites for antinociceptive activity. The periaqueductal gray region (PAG) of the midbrain was the most sensitive site examined for both morphine and La^{3+}. This site is reportedly also the brain region of greatest opiate receptor concentration (46).

These results offer strong evidence that the acute analgesic effects of both morphine and La^{3+} may be related to reduced flux and/or binding of Ca^{2+} in brain tissue, especially in the PAG region. There was not, how-

ever, complete similarity between the effects of morphine and La^{3+} in producing analgesia. Thus pretreatment of the animals with pargyline or cAMP modified the antinociceptive effect of morphine but not of La^{3+}. Another difference was that interactions between La^{3+} and morphine, naloxone, or Ca^{2+} appeared to be of a noncompetitive nature because all three agents markedly decreased the slope of the La^{3+} dose-response curve, whereas the slope of opiate agonist dose-response curves was not significantly altered by these substances. Furthermore, naloxone antagonized La^{3+} analgesia only when injected prior to the La^{3+} injection. Finally motor activity in mice was decreased by i.c.v. La^{3+} injection but increased by morphine administration.

The antagonistic effects of Ca^{2+} and reserpine toward pethidine analgesia were found to be additive when Ca^{2+} was administered after reserpine, but were reversed when Ca^{2+} was given first (37). In a similar study using morphine-tolerant mice (47), it was reported that EGTA reversed the antagonism by reserpine, causing a net decrease in morphine AD_{50} compared with control-tolerant mice. Conversely, Ca^{2+} increased the effect of reserpine in tolerant mice such that the LD_{50} was exceeded before the tailflick AD_{50} could be determined. An involvement of Ca^{2+} in the effects of reserpine in antagonizing morphine analgesia was inferred.

Ca^{2+}, Mg^{2+}, and Mn^{2+} also antagonize naloxone-reversible acetylcholine analgesia (48) and analgesia due to β-endorphin and methionine-enkephalin (D. B. Chapman and E. L. Way, unpublished observations). Analgesia was potentiated by EGTA, while the ionophore A23187 was used in both cases to increase the antagonist potency of Ca^{2+}. Antinociceptive effects produced by androsterone sulfate and Na_2SO_4 (49) were interpreted in terms of Ca^{2+} antagonist properties of these agents. A number of reports (50–53) have shown that i.c.v. injections of the hormone calcitonin result in analgesia. At the same time a decrease in blood Ca^{2+} was observed (50) and this analgesia is thus presumed to be due to the hormone's ability to reduce Ca^{2+} levels in the body. Analgesia was not, however, reversed by the antagonist levallorphan (53).

In contrast to preceding results, Vocci et al (54) demonstrated a dose-dependent Ca^{2+} potentiation of dibutyryl cGMP analgesia after i.c.v. injections of the metal ions. The usual Ca^{2+} antagonism of morphine analgesia was also reported. Mg^{2+} produced a dose-dependent inhibition of dibutyryl cGMP analgesia but did not affect morphine analgesia, while Ba^{2+} had additive effects with db cGMP and to some extent also with morphine. EDTA inhibited db cGMP without altering morphine effects, while EGTA had no effect on db cGMP analgesia but potentiated morphine analgesia. In a companion report (55) it was shown that subconvulsive doses of Ba^{2+} had antinociceptive actions in the mouse; this effect of Ba^{2+} presumably

accounted for the effects of Ba^{2+} on db cGMP and morphine analgesia described above. The Ba^{2+}-induced analgesia peaked 10 min after injection and was antagonized by prior administration of atropine sulfate or naloxone.

Copper ions have also been implicated in morphine antinociception although with opposite effects to those of Ca^{2+}. Stern (56) and Kaneto et al (57) found enhanced morphine analgesia in mice after intraperitoneal and s.c. administration of doses of $CuCl_2$ respectively. Also diethyldithiocarbamate (DDC), an inhibitor of the copper-containing enzyme dopamine β-hydroxylase (DBH), was found to potentiate morphine analgesia (58). Since the effect did not seem to be related to changes in brain dopamine or noradrenaline content, the authors postulated that the DDC might be acting as a copper chelator to produce its effect on morphine antinociception. Thus in a second report (59) copper levels in plasma were examined after drug treatments. It was found that while acute morphine administration in rats caused a depletion of plasma copper levels, a dose of DDC which significantly potentiated morphine analgesia, caused no further decrease in plasma copper levels. In morphine-tolerant animals a marked increase in plasma copper content was noted which was partially reversed on morphine withdrawal. Administration of DDC to tolerant rats resulted in an increased level of analgesia and in a significant decrease in the elevated copper level. The authors concluded that the alterations in copper levels observed may be directly related to morphine analgesia and tolerance. In contrast to these results, Reinhold et al (60) found that DDC has no effect on morphine analgesia in the rat. Bhargava & Way (61) found similar potentiation of morphine analgesia using 1-phenyl-3(2-thiazolyl)-2-thiourea (PTT), another DBH inhibitor. PTT administration also partially inhibited morphine tolerance and dependence development in the mouse. Unlike the report of Iwata et al (59), no changes were observed in copper levels (in this case brain copper levels) after PTT or acute morphine treatment, although some increase in brain copper did occur after three repeated injections of PTT. The authors observed that if PTT were acting as a copper chelator then an antagonism rather than an enhancement of morphine analgesia might be expected, since copper potentiates morphine analgesia.

Bhargava (62) has since reported that i.c.v. injection of copper sulfate failed to potentiate morphine analgesia in naive mice, although analgesia was significantly enhanced in tolerant-dependent animals.

Recently it has been reported (63) that i.c.v. injection of Cu^{2+} produced analgesia in mice. This effect was naloxone reversible. Other researchers have also observed Cu^{2+}-induced antinociception after i.c.v. injection although in this case the effect was not found to be naloxone reversible (D.B. Chapman and E. L. Way, unpublished observations). The Cu^{2+} analgesia

was reversed by Ca^{2+}, however, and was also progressively reduced with increasing levels of morphine-tolerance development. Since brain Ca^{2+} levels increase during tolerance development (see later), these data are consistent with a Ca^{2+} antagonist effect of Cu^{2+} in producing analgesia. Pretreatment of animals with penicillamine, a drug used clinically to reduce brain Cu^{2+} levels in patients with Wilson's disease (64), resulted in a significant inhibition of morphine and β-endorphin analgesia. This suggests a role for Cu^{2+} in acute narcotic effects.

One report has appeared suggesting that lithium ions are able to antagonize morphine-induced analgesia (65).

METAL ION EFFECTS ON TOLERANCE-DEPENDENCE DEVELOPMENT

The first report implicating calcium in the actions of narcotic drugs appeared in 1936. In this study (66) guinea pigs that had been preinjected with calcium gluconate (0.25 g, s.c.) were found to develop less analgesic tolerance to daily morphine injections than animals treated with morphine alone. Conversely, animals treated with the cation chelator oxalic acid showed slightly enhanced tolerance development. Five years later Detrick & Thienes (67) reported that rats kept on a high calcium diet and given parathyroid hormone injections during morphine tolerance induction subsequently showed few if any signs of withdrawal. Animals kept on a low calcium diet displayed the irritability typical of morphine withdrawal.

Kakunaga et al (68) examined the effects of Ca^{2+} on tolerance development using intracisternal injections of the cation. Mice receiving repeated injections of morphine plus Ca^{2+} were significantly less tolerant to the analgesic effects of morphine than those receiving morphine alone. More recently it was observed (38) that a single i.c.v. injection of Ca^{2+} immediately prior to morphine pellet implantation did not significantly alter tolerance development compared with animals treated with morphine alone. This was presumably because Ca^{2+} levels became too low to affect morphine analgesia between 6–24 hr after injection (38). No change in morphine tolerance (compared with control-tolerant animals) was seen in animals receiving constant i.c.v. infusion of $CaCl_2$ during tolerance development, using Alzet osmotic minipumps (69). The dose of Ca^{2+} infused was only 2 μmol/24 hr and the author opined that higher doses should be examined. On the other hand, chronic i.c.v. infusion of EGTA resulted in greater morphine tolerance compared with saline-infused controls.

Rats that had received large quantities of calcium gluconate in their drinking water during tolerance development and that were injected with Ca^{2+} prior to naloxone-induced withdrawal showed fewer abstinence signs than saline-maintained controls (70).

Bhargava (62) investigated the effects of i.c.v injections of Cu^{2+} on abstinence signs in morphine-dependent mice. Cu^{2+} significantly inhibited the naloxone-precipitated jumping response but did not affect other signs of withdrawal. When abstinence was precipitated with the partial antagonist nalorphine, neither Cu^{2+} nor Ca^{2+} affected withdrawal signs (including jumping).

METAL ION EFFECTS ON NONANALGETIC ACTIONS OF NARCOTICS

A number of reports have appeared which suggest that Ca^{2+} is also involved in actions of narcotics other than those mediating analgesia.

Takemori (71, 72) reported that morphine inhibited potassium-stimulated respiration in cerebral cortical slices from rat brain. It was later shown that this inhibitory effect of morphine could be demonstrated only in calcium-free Ringer's solution (73). These results were later confirmed (74).

Nutt (36) confirmed that Ca^{2+} antagonizes morphine antinociception and also observed that Ca^{2+} noncompetitively antagonizes the inhibition by morphine of guinea pig ileum contractions produced by coaxial stimulation. Comparable concentrations of Mg^{2+} were without effect. Morphine inhibition of the effects of acetylcholine and serotonin on the guinea pig ileum and the depression of acetylcholine output by morphine were not reversed by Ca^{2+}. Heimans (75) also reported Ca^{2+} antagonism of morphine effects in the transmurally stimulated guinea pig ileum. In a very recent paper (76) it was reported that Ca^{2+} dose dependently diminishes the inhibitory effects of morphine on stimulated guinea pig ileum contractions. Data analysis suggested a competitive antagonism between Ca^{2+} and morphine in contrast to the earlier report (36). Data from this laboratory support the findings by Opmeer and van Ree (J. P. Huidobro-Toro, personal communication).

The ability of the narcotic levallorphan to inhibit growth of cultures of the bacteria *Escherichia coli* decreases with increasing concentrations of Mg^{2+} or Ca^{2+} in the medium (77).

Sullivan & Wong (78) have reported that the myocardial depressant effect of morphine may be due to its interference with Ca^{2+} transport. Using an in vitro rabbit heart preparation it was observed that depression was enhanced by Ca^{2+} deficiency in the perfusate, but was antagonized by increased Ca^{2+} concentrations. In a continuation of these studies the antagonist effect of morphine on the positive inotropic effect of ouabain in the isolated rabbit heart was examined (79). This effect of ouabain is associated with an increased flux of Ca^{2+} across membranes. Decreased Ca^{2+} in the medium resulted in increased depression by morphine.

Morphine, *l*-cyclazocine, and *l*-pentazocine cause contraction of the rat aortic strip (80) in a manner suggesting an increased Ca^{2+} influx into the muscle. It was later shown (81) that contractions caused by *l*-pentazocine and K^+ can be antagonized by methadone and *l*-acetylmethadol; these latter agents apparently acted as Ca^{2+} antagonists, for their effects were reduced with increasing Ca^{2+} concentration. It was also reported, however, that the rat aortic strip does not appear to possess classical opiate receptors, that there was no correlation between the vascular and analgesic effects of these drugs, and that naloxone was not an effective antagonist. This may indicate that these results are not directly related to Ca^{2+} antagonism of narcotic analgesia, since analgesia does involve opiate receptor occupation and is naloxone reversible. The inhibitory effects of these agents on the rat aortic strip seem best correlated with their oil-water partition coefficients.

Ca^{2+} antagonism of morphine-induced respiratory depression (as well as morphine analgesia) has been reported (82), although Ca^{2+} and Mn^{2+} were without effect on β-endorphin–induced body temperature changes (83). Conversely, lithium ions have been reported to reverse morphine effects on temperature (84).

EFFECTS OF NARCOTIC TREATMENT ON BODY METAL ION CONTENT

As a consequence of this work showing probable involvement of metal ions in narcotic actions, a number of investigators have studied the effects of opiates on metal ion levels in the body. Lowered brain Ca^{2+} levels were observed after a single, very high (100 mg/kg) dose of morphine (85), while lower doses were without effect. The maximum change was seen 30 min after injection of morphine. The effect decreased with repeated morphinization and was no longer seen in tolerant animals. In addition, reciprocal changes in brain magnesium ion concentration were found after a single dose of morphine (29). A dose-dependent, nalorphine-reversible hypermagnesemia was also reported after intravenous morphine administration (86).

In the first two of a series of papers by Ross and colleagues, it was reported (87, 88) that a single dose of morphine sulfate (6–25 mg/kg) produced significant (28–44%) dose-dependent decreases in tissue Ca^{2+} content in all of eight separate rat brain regions examined. Using fairly large doses (25–50 mg/kg), this effect was found to be time-dependent, stereoselective (with levorphanol effective and dextrorphan ineffective), and antagonized by naloxone. However, the degree of Ca^{2+} reduction is extraordinarily high and difficult to interpret, particularly because all brain regions were decreased equally. Furthermore, other investigators have failed to detect

significant changes in whole brain Ca^{2+} levels after similar treatment (R. A. Harris, personal communication; H. W. McCain, personal communication). Alcohol was reported to produce a comparable decrease which was also naloxone reversible. Reserpine also depleted brain Ca^{2+} levels but in a non-naloxone–reversible fashion, while pentobarbital was without effect. When a large dose of reserpine was followed by a large dose of morphine the Ca^{2+}-depleting effects of these drugs were additive, resulting in a greater than 65% decrease in all eight brain regions. Subsequently, a rapid development of tolerance to the Ca^{2+}-depleting effects of a single dose of morphine (25 mg/kg) was demonstrated lasting up to 7 days (89, 90). The induction of tolerance was prevented by pretreatment with the protein synthesis inhibitor cycloheximide. Reserpine did not produce tolerance to its own Ca^{2+}-depleting effects, nor did reserpine pretreatment affect morphine-tolerance development.

The effects of narcotics on the subcellular distribution of metal ions in rat brain were also examined (91, 92). Ca^{2+} depletion after acute morphine treatment was confined to the synaptosomal particulate fraction of cerebral cortices, while no effect was seen in any other fraction including myelin, mitochondria, mitochondrial lysate, nuclear, crude microsomal, and soluble fractions. It is of interest that opiate receptor binding was reported to occur mainly in synaptosomal fractions (93). The same dose of morphine failed to cause any detectable changes in the levels of Na^+, K^+, or Mg^{2+}.

Harris et al (94) and Yamamoto et al (95) found that acute treatment with morphine (25 mg/kg) 30 min prior to sacrifice resulted in a significant depletion (26–29%) in unlysed synaptosomal calcium content in rats but not in mice. The Ca^{2+} content of other subcellular fractions was not altered. These workers also found that when ^{45}Ca was injected i.c.v. 6 hr prior to sacrifice in order to label brain Ca^{2+} pools (94–96) significant decreases in mouse brain synaptic vesicle content of ^{45}Ca following morphine injection resulted. In light of these results Harris and co-workers suggested that acute morphine actions may involve interference with neurotransmitter release, since transmitter release is probably regulated by Ca^{2+}, while synaptic vesicles contain high levels of neurotransmitters.

In contrast to this acute reduction in brain Ca^{2+} levels, chronic morphine treatment of both rats and mice resulted in significant increases (up to 100%) in synaptosomal Ca^{2+} content (94–98). Simultaneous chronic administration of naloxone blocked this effect (95) while naloxone precipitated withdrawal in dependent animals quickly reduced the elevated Ca^{2+} levels toward control values (94–97). The increased Ca^{2+} levels were observed to occur in the synaptic vesicle fractions by Harris et al (94, 96) and in the synaptic plasma membrane and synaptic vesicle fractions by Ross (97).

In vitro ^{45}Ca binding studies using synaptic membranes and synaptic vesicles showed that prior acute morphine treatment resulted in an increase in high affinity (10^{-7} to 10^{-5} M) ^{45}Ca binding sites on synaptic plasma membranes, while chronic treatment caused a decease in binding sites. Low affinity (10^{-3} M) binding sites on synaptic vesicles were similarly altered (95, 99). These results were interpreted as acute morphine treatment causing a reduction in brain Ca^{2+} levels (and hence an increase in ^{45}Ca binding sites) while chronic treatment resulted in increased Ca^{2+} levels (and hence a decrease in ^{45}Ca binding sites). Binding to intact synaptosomes was not altered, leading the authors to propose that binding sites on the inner surface of synaptic membranes were being affected. In a similar study Ross (97) measured low, intermediate, and high level ^{45}Ca binding to synaptic membranes (but not vesicles) from chronically treated animals. Again, chronic treatment resulted in decreased ^{45}Ca binding capacity, although in this case both low and high affinity binding was reduced. Similar results were seen for SPM-1, SPM-2, and SPM-3 fractions (32).

Recently it has been shown that β-endorphin and methionine-enkephalin also deplete the Ca^{2+} content of synaptic membranes and synaptic vesicles (32).

In contrast to these results Sanghvi & Gershon (70) reported that morphine-dependent rats had significantly lowered brain Ca^{2+} levels. However, in actuality the Ca^{2+} levels were determined after naloxone administration, and hence this finding would appear to be an antagonist-precipitated abstinence effect akin to those reported above (94–97). A reduced brain Mg^{2+} content was also found in chronically treated rats after naloxone-precipitated abstinence, an effect that was blocked by prior Ca^{2+} administration (100).

The results of opiate administration on brain Ca^{2+} levels suggest that while acute treatment results in decreased Ca^{2+} levels (and perhaps altered neurotransmitter release), a homeostatic response occurs during chronic treatment which results in increased Ca^{2+} levels and may directly result in tolerance-dependence development.

Since agents that reduce Ca^{2+} influx into cells (La^{3+}, EGTA, and EDTA) were themselves capable of producing analgesia (as discussed previously) and since acute morphine treatment results in decreased synaptosomal Ca^{2+} content, Schmidt & Way (101) hypothesized that the increased synaptosomal Ca^{2+} content observed during narcotic tolerance development should be associated with a hyperalgesic response. Indeed, a highly significant hyperalgesic response was observed in morphine-dependent mice 12 hr after removal of morphine pellets. The effect was enhanced by i.c.v. injections of Ca^{2+}, Mg^{2+}, or Mn^{2+}. In fact, morphine-dependent mice were more than twice as sensitive to Ca^{2+}-induced hyperalgesia than placebo-treated

controls. Conversely, morphine-dependent mice were only half as sensitive to EGTA-induced analgesia, presumably as a result of their higher synaptosomal Ca^{2+} content.

A number of papers have appeared in which narcotic-induced changes in metal ion content of body regions other than the brain are reported. Morphine treatment causes antidiuresis, and tolerance to this effect develops with repeated morphinization. Increased urinary excretion of Na^+ and K^+ was found after acute morphine treatment, but no change from control levels in morphine-tolerant animals (102). It was later reported that increased urinary sodium, potassium, phosphate, calcium, and urea excretion accompany diuresis in morphine-tolerant rats (103). Nalorphine partly reversed the hypercalciurea and urea diuresis. Increased Ca^{2+} content was observed in adrenal cells after acute morphine treatment, but the Ca^{2+} content was decreased after chronic treatment (104). De Boer & Tofano (105) reported increased blood K^+ in rats after acute treatment with very high levels of morphine (100 mg/kg), while serium ionized calcium levels in newborn infants showing withdrawal symptoms (due to maternal narcotic addiction) were significantly lower than those in normal control infants (106).

Serum and urine levels of Mg^{2+} in rats were increased by acute morphine treatment (100) while only urinary Mg^{2+} was increased after chronic treatment.

As discussed previously, changes in plasma copper levels have been noted after acute (decreased levels) and chronic (increased levels) morphine treatment (59).

EFFECTS OF OPIATES ON CALCIUM BINDING AND CALCIUM FLUXES

A likely explanation for the effects of metal ions (and particularly Ca^{2+}) on narcotic actions and the narcotic-induced changes in brain levels of Ca^{2+} is that the drugs alter metal ion (Ca^{2+}) binding and/or fluxes.

Mulé (107) demonstrated that narcotic analgetics (as well as other CNS active drugs) inhibited Ca^{2+} binding to phospholipids in vitro. However, inconsistencies appeared when analgesic potency was compared with Ca^{2+} binding inhibition. Thus naloxone and dextrorphan were more effective inhibitors than morphine, and it was concluded that effectiveness was related to the degree of drug ionization. Later Mulé (108) speculated that opiates might compete with Ca^{2+} for anionic binding sites on membrane phosphatides. Thus assuming that phospholipids act as CNS receptors for narcotic drugs, the binding of opiates to phospholipids in this manner could lead to alterations in ion conductance, membrane permeability, electrical

activity, and neuronal excitability; these changes in turn produce opiate effects such as analgesia. It was reported that morphine inhibited ^{45}Ca binding to purified bovine gangliosides, while nalorphine exhibited a biphasic effect, enhancing binding at low concentrations and antagonizing binding at high concentrations. The effect of morphine was partally inhibited by nalorphine (109).

In another study (110) of this type, the ability of phosphatidyl serine (PS) to enhance ^{3}H-dihydromorphine binding to synaptic membranes was reduced using either the Ca^{2+} salt of PS or using PS in the presence of 1 mM Ca^{2+}. Only slight enhancement was seen with Ca^{2+}-PS and none at all in the presence of Ca^{2+}. However, as the authors pointed it was not possible to determine whether the inhibition was due to Ca^{2+} occupying an anionic receptor site independently of the lipid or whether it complexed directly with the PS and thus produced its effect. The effects of morphine on the incorporation of ^{14}C-serine into membrane phospholipids via Ca^{2+}-stimulated base exchange were examined (111) in order to investigate the possibility that narcotic/Ca^{2+} interactions might result in measurable changes in brain membrane phospholipid composition. Acute and chronic treatment decreased basal-exchange in the absence of Ca^{2+}, while chronic but not acute treatment increased Ca^{2+}-stimulated exchange. Some stimulation of ^{14}C-ethanolamine exchange and a decrease of ^{14}C-choline exchange was seen with chronic treatment. In vitro treatment increased the basal rate of ^{14}C-serine exchange in a naloxone reversible fashion.

Levorphanol has been observed to inhibit high affinity ^{45}Ca binding to synaptic membranes in vitro in a dose-dependent manner (31, 112). The inhibition was noncompetitive and blocked by naloxone. Moreover it was reasoned that cooperative Ca^{2+} binding was being inhibited since increasing concentrations of levorphanol changed the shape of the Ca^{2+} binding curves from sigmoid to hyperbolic. It was thus suggested that Ca^{2+} and opiate receptor sites are adjacent and may be functionally linked through subunit interactions. Pentobarbital and chlorpromazine also caused decreases in Ca^{2+} binding but this effect was not reversed by naloxone. Similarly, β-endorphin is also reported to be a potent inhibitor of Ca^{2+} binding (32). In contrast to these results it was found that in vitro addition of morphine HCl in concentrations up to 10^{-5} M did not alter high (10^{-7} M) or low (10^{-3} M) affinity ^{45}Ca binding to synaptic membranes (95).

Terbium binding (as measured by protein-sensitized fluorescence of Tb^{3+}) was used to detect increased cationic binding sites (and hence it was reasoned, decreased Ca^{2+} content as discussed in the previous section) on synaptic membranes after acute morphine treatment (113). Enzyme treatment suggested that the Ca^{2+} receptor may be a protein-glycolipid complex. In a similar investigation, in vivo and in vitro morphine treatment was found to be without effect on Tb^{3+} binding to synaptic vesicles (114).

In the first examination of narcotic effects on Ca^{2+} fluxes, it was reported that morphine (10^{-3} M) inhibited K^+ (and EDTA) stimulated ^{45}Ca influx into and efflux from rat brain slices (115). This effect was partially reversed by nalorphine. An interesting aspect of the results was that morphine only inhibited influx into slices in low-Ca^{2+} medium, suggesting a direct effect on Ca^{2+} movements, rather than the effects on Ca^{2+} being secondary to some other mechanism of action of the drug. Later Kaku et al (116) reported that acute in vivo or in vitro morphine treatment caused a decreased ^{45}Ca uptake into mouse synaptosomal fractions. No effect was seen with chronically treated animals. ^{45}Ca uptake (binding?) by the synaptic membrane fraction was not affected by morphine.

More recently it was shown that acute in vivo or in vitro administration of morphine caused a dose-dependent decrease in both the initial rate and maximum level of spontaneous ^{45}Ca uptake into synaptosomes (117, 118). This effect could be reversed by naloxone. In vitro treatment with levorphanol similarly inhibited ^{45}Ca influx. Data analysis indicated that morphine caused noncompetitive inhibition of ^{45}Ca uptake. Conversely, chronic morphine treatment in vivo caused progressive increases in synaptosomal ^{45}Ca uptake as tolerance developed. It has also been reported that increased ^{45}Ca efflux from synaptosomes was observed in tolerant animals. This effect was naloxone-reversible, while acute in vivo and in vitro treatment with morphine or levorphanol had no effect on ^{45}Ca efflux (119).

In similar studies using rabbit synaptosomes, levorphanol in vitro reduced K^+-stimulated ^{45}Ca uptake in a naloxone-reversible fashion (31). This effect [in contrast to (115)] was seen at different Ca^{2+} concentrations and was apparently due to noncompetitive inhibition with no significant change in K_m values. Chronic treatment of mice with levorphanol resulted in increased K^+-stimulated ^{45}Ca uptake compared with control animals. In contrast to the results above (117, 118) no effect was seen with resting levels of flux at low K^+ levels (32). End et al (120) have also studied ^{45}Ca uptake into synaptosomes in the presence of morphine. K^+-stimulated influx was inhibited by the drug in a naloxone-reversible fashion, but again in contrast to the above reports, morphine had no effect on basal ^{45}Ca uptake.

EFFECTS OF METAL IONS ON OPIATE BINDING

Parallel to the effects described in the last section, a number of reports indicate that metal ions have pronounced effects on opiate binding. Thus Na^+ enhances stereospecific opiate antagonist binding while reducing agonist binding (121–124). Similar results have been reported by other investigators (125–128). Lithium ions have a similar but less pronounced effect (125, 129) and in one report were without effect (128). One possible result

of this Na^+ effect in vivo might be that the binding of an opiate agonist to its receptor would regulate (decrease) Na^+ binding in the same vicinity and thus perhaps alter Na^+ fluxes. This condition would then be reversed by antagonist binding which would result in the receptor having a higher affinity for Na^+. In support of this concept, it was reported that opiates may impair Na^+ influx at postsynaptic membranes (130). However, Cardenas & Ross (92) failed to detect any change in Na^+ content in different subcellular fractions after acute morphine treatment. It can always be argued, however, that the technique employed lacked sufficient sensitivity to detect any small changes which may have occurred.

Divalent metal ions also influence in vitro opiate binding. Pert & Snyder (123) observed that both Ca^{2+} and Mg^{2+} inhibited naloxone binding although they later reported Ca^{2+} to be without effect at physiological levels (129). Hitzemann et al (125) also examined naloxone binding in the presence of divalent cations and found Ca^{2+}, Mg^{2+}, and Mn^{2+} to have inhibitory effects. The inhibitory effect of levorphanol on naloxone binding also appeared to be blocked by these cations. Also Ca^{2+} has been reported to produce a marked inhibition of dihydromorphine binding to synaptic membranes (110).

Conversely it has been demonstrated that Mg^{2+}, Mn^{2+}, Ca^{2+}, and Ni^{2+} are capable of enhancing opiate agonist binding with Mn^{2+} having the most, and Ca^{2+} the least, marked effect (131). At the same concentrations the ions had little or no effect on antagonist binding. In contrast Cu^{2+} and Fe^{2+} lowered agonist binding more than antagonist binding. All of these effects were most pronounced in the presence of Na^+, and it was suggested that Mn^{2+} acts by altering receptor sensitivity to Na^+. Mn^{2+} also enhanced the ability of unlabeled agonists to inhibit ^{3}H-naloxone binding. Furthermore, EDTA but not EGTA reduced dihydromorphine binding in the presence of Na^+, while naloxone binding was not affected. The EDTA effect was reversed by Mn^{2+} or Mg^{2+}. From these results the authors concluded that calcium was probably not the endogenous divalent cation which regulates the opiate receptor. The ability of Cu^{2+} to inhibit agonist binding is apparently a result of Cu^{2+} binding to SH groups on membranes at the active site, since the inhibitory effects of Cu^{2+} are abolished after incubation with SH reductants (63). Blume (132) has reported that Mn^{2+} decreases the rate of dissociation of membrane-bound dihydromorphine, etorphine, and naltrexone. Furthermore, Mn^{2+} was found to antagonize the ability of Na^+ to increase dissociation.

It has been postulated that the Na^+ effect is due to Na^+ induced displacement of Ca^{2+} from synaptic tissue, while Mn^{2+} also acts to displace Ca^{2+} and thus retain the opiate receptor in a divalent ion–associated state, easily recognizable by opiate agonists (31, 32). Ross (31, 32) further draws from

results of Cho et al (133) in which cerebroside sulfate (CS) was treated as a model opiate receptor and in which agonist CS complexes were reported to be more hydrophobic (facilitated by divalent cations) and antagonist CS complexes more hydrophilic (facilitated by monovalent cations). Ross (31, 32) thus proposed that agonists bind more readily to the divalent cation form of receptor and antagonists to the monovalent form. To further support this contention, experiments have been performed which show that naloxone partially reverses Mn^{2+} inhibition (but not Na^+ inhibition) of Ca^{2+} binding to synaptic membranes (31, 32). Thus it is argued that Mn^{2+} produces a Ca^{2+}-associated type of receptor conformation which is reversed by naloxone to a more hydrophilic form. Furthermore, evidence from the same group indicates that *n*-ethylmaleimide treatment of membranes results in a significant reduction in Ca^{2+} receptor binding. This effect was inhibited by naloxone, again suggesting that in some way antagonist binding to the opiate receptor alters the Ca^{2+}-associated nature of the nearby membrane (134).

Divalent cation enhancement of opiate agonist binding in vitro (131) would seem to imply that these ions should also enhance opiate agonist activity in vivo. However, as previously described, opposite effects have been reported. One possible explanation for this might be that in binding to their receptors, opiate agonists preferentially abstract divalent metal ions from other membrane sites where they had served as enzyme modulators. Thus agonist binding would directly alter enzyme activity. The addition of excess divalent metal ions in vivo would reverse the changed enzyme activity and thus antagonize the effects of the opiates. It is of interest in that whereas Mn^{2+} and Mg^{2+} and perhaps Ca^{2+} all increase opiate agonist receptor binding in vitro, and antagonize opiate analgesia in vivo, copper ions tend to potentiate opiate analgesia and yet antagonize stereospecific opiate receptor binding in vitro. Whether this apparent relationship is of any significance is not known.

The reduction of agonist binding in the presence of Na^+ may be due to conformational changes in the opiate molecule induced by the metal ions. The possibility of such interaction between Na^+ and K^+ and methionine-enkephalin was investigated using circular dichroism to detect any conformational changes (135). Marked differences were detected between the conformational behavior of the peptide in the presence of Na^+ and K^+. Thus although the Na^+/methionine-enkephalin interaction was weak, it was pointed out that due to the large excess of Na^+, such conformational changes might well contribute to the reduced agonist binding. The fluorescence of morphine is altered by Ca^{2+} and Mg^{2+} (136), suggesting complex formation between morphine and the ions. Such an effect might contribute to the observed modifications of agonist activity and binding by these ions.

OPIATE EFFECTS ON SPECIFIC ENZYME SYSTEMS

Much evidence has thus been accumulated which implicates metal ions, and especially divalent metal ions, in the actions of narcotic drugs, both by metal ion–induced modification of drug actions and by gross changes in bodily disposition of metal ions caused by narcotics. As a consequence, attempts have been made to locate the precise site or sites of these drug/metal ion interactions and interest has naturally centered around enzyme systems whose functions involve metal ions and especially Ca^{2+}.

ATPase

ATPase enzymes are important in maintaining active ion transport systems and as such have been a focus of attention as possible sites of narcotic actions in view of opiate effects on Ca^{2+} disposition. Acute morphine treatment caused increased ATPase activity (and increased Ca^{2+} content) in adrenal cells, while chronic treatment caused a depression in enzyme activity (and decreased Ca^{2+} content). The acute treatment also caused a decrease in neurotransmitters (104). Morphine inhibited total ATPase activity in rat brain microsomes in the absence of Na^+, K^+, and Mg^{2+} (137), while Kaku et al (116) reported inhibition of mouse brain synaptosomal Mg^{2+}-Ca^{2+}-ATPase after acute in vivo or in vitro treatment. Interestingly, ^{45}Ca uptake into synaptosomes was similarly inhibited, but neither ATPase activity nor ^{45}Ca uptake was altered compared with controls in chronically treated animals. Synaptic membrane ATPase was affected by drug treatment in a similar fashion, suggesting a membrane-located site of narcotic action. In contrast to these results, morphine stimulation of Na^+-K^+-stimulated, and Mg^{2+}-dependent ATPase activity was reported (138).

Later Desaiah & Ho (139) observed significant increases in oligomycin-insensitive Mg^{2+}- and Na^+-K^+-ATPase activities in the crude mitochondrial fraction of mouse brain after acute morphine treatment. In vitro morphine treatment had no effect. The acute effect was antagonized by naloxone administered 15 min after morphine, while in animals receiving naloxone immediately followed by morphine a significant inhibition in Na^+-K^+-stimulated and oligomycin-sensitive Mg^{2+}-ATPase was observed. In morphine-tolerant mice a further increase in Na^+-K^+-stimulated, and oligomycin-insensitive ATPase activities was observed, as compared to acutely treated animals. These authors further reported that both dopamine and norepinephrine enhanced Na^+-K^+-ATPase activity in naive but not in morphine-tolerant mice. In a subsequent dopamine-sensitive ATPase activity in vitro. Acute and chronic administration of morphine also inhibited

catecholamine-sensitive ATPase activity. Na^+-K^+-ATPase activity was more affected by morphine than Mg^{2+}-ATPase activity.

Increased Mg^{2+}-dependent ATPase activity was observed in mouse brain synaptic vesicles from animals treated chronically with morphine (141). Mg^{2+}-dependent ATPase and Na^+-K^+-stimulated ATPase activities of synaptic plasma membrane (SPM) from morphine-tolerant mice were not significantly different from placebo-treated control animals.

Recently it has been shown that while acute levorphanol treatment produced no significant change in synaptic membrane Ca^{2+}-ATPase activity, chronic treatment resulted in a significant loss of activity (32). In a preliminary communication (142) it has been reported that morphine in vitro stimulated synaptosomal membrane Ca^{2+}-ATPase but inhibited Ca^{2+} binding to a Ca^{2+}-dependent activator of the ATPase.

Although the differing effects of narcotics on ATPase activities described here are difficult to reconcile, the consistently positive results in these experiments do suggest that ATPase's may be important sites of opiate drug actions. The differences in ATPase activity in acutely and chronically treated animals seen in several of these experiments may represent homeostatic mechanisms tending to overcome the drug effects. Mg^{2+}-dependent ATPase is believed to be involved in the regulation of neurotransmitter release (143, 144), and thus changes in enzyme activity by narcotics could directly affect neurotransmission. Ca^{2+} is also implicated in neurotransmitter release, while Mg^{2+}-ATPase is apparently involved in Ca^{2+} accumulation by synaptic vesicles (145). Changes in ATPase activity by narcotics might thus give rise to the changes in Ca^{2+} content noted previously. The increased Mg^{2+}-dependent ATPase activity in chronically treated animals observed by Yamamoto et al (141) might therefore be directly responsible for the increased vesicular Ca^{2+} content observed after chronic morphinization (94–97). It was also postulated that the decrease in Ca^{2+}-ATPase activity described above (32) could result in the increased Ca^{2+} levels seen in synaptosomes after chronic opiate treatment. These would thus represent homeostatic mechanisms for maintaining neuronal activity.

Adenylate Cyclase

Adenylate cyclase represents another likely site for narcotic/metal ion involvement, since the enzyme is regulated by Ca^{2+}. The increase in intracellular Ca^{2+} which occurs when the adenylate cyclase system is activated may arise from an increased uptake of extracellular Ca^{2+}, by release of bound intracellular Ca^{2+}, or by both mechanisms. It has been proposed that Ca^{2+} influx results in the formation of a Ca^{2+}-CDR (Ca^{2+}-dependent regu-

lator) complex (146), which then activates adenylate cyclase to produce cAMP, and concomitantly to activate phosphodiesterase to decrease cAMP so produced (147). Kakiuchi et al (148) have proposed that the phosphodiesterase may have a greater affinity for cGMP, resulting in an altered intracellular cAMP/cGMP ratio. Opiates with their ability to change Ca^{2+} fluxes may thus modulate these enzyme effects.

Sharma et al (149) have shown that acute administration of opiates in a neuroblastoma X glioma cell line caused inhibition of adenylate cyclase activity while chronic exposure led to increased enzyme activity. These changes are mirrored by similar changes in Ca^{2+} levels after in vivo narcotic administration as discussed previously. Possibly the opiate-induced changes in Ca^{2+} disposition are responsible for the changes in adenylate cyclase activity. In support of this theory it has been reported that physiological concentrations of Ca^{2+} are necessary for opiates to have an inhibitory effect on adenylate cyclase activity (150, 151). Recently morphine has been shown to inhibit Ca^{2+}-dependent activation of adenylate cyclase in neuroblastoma X glioma hybrid cells (152). In another report (153) opiates were found to inhibit hormone-induced increases in cAMP in cell lines even in the absence of Ca^{2+} in the external medium. In other experiments, however, short-term and chronic exposure of the cells to low Ca^{2+} concentrations mimicked the effects of opiate treatment on cAMP levels. Guanylate cyclase is also regulated by Ca^{2+} (19) and thus it too represents a possible locus for narcotic actions involving divalent metal ions since opiates have been shown to produce changes in cellular cGMP content (154–157). The increase in cGMP content observed by Minneman & Iversen (158) was apparently involved with Ca^{2+} changes in some way, since no effect was observed in the absence of Ca^{2+} from the external medium.

Other Enzyme Systems

Other relevant enzyme systems which may be affected by narcotics via changes in Ca^{2+} disposition are tyrosine hydroxylase and protein kinase. Tyrosine hydroxylase activity is regulated by Ca^{2+} (23) and is reportedly altered by narcotic administration (159, 160). Recently, chronic morphine treatment has been shown to attenuate the effect of the ionophore A23187 on tyrosine hydroxylase activity in synaptosomes (161). Ca^{2+} also regulates synaptic membrane-bound protein kinase activity (21, 22) as do cyclic nucleotides (162–165), while narcotic administration has been reported to alter protein kinase–induced phosphorylation of proteins (166–168). Ca^{2+} stimulation of phosphorylation in vitro was inhibited at a high dose (10^{-3} M) of morphine. This appeared to be a nonspecific effect as it was not reversed by naloxone (167). Methadone and morphine (at high concentrations) have been shown to inhibit A23187-stimulated phosphorylation in rat

striatal synaptosomes (169), while morphine enhanced the effects of Ca^{2+} and A23187 in synaptosomes from tolerant-dependent mice (161).

OPIATE/CALCIUM EFFECTS ON NEUROTRANSMITTER RELEASE

Since the end-point of narcotic action is presumably via altered release of neurotransmitters, a number of investigators have examined the effects of Ca^{2+} on opiate inhibition of neurotransmitter release. Shikimi et al (170) reported that morphine at a high concentration (1 mM) inhibited K^+-stimulated acetylcholine release from mouse cerebral cortical slices in normal medium but not in Ca^{2+}-free medium.

It has since been shown that s.c. injections of Ca^{2+} in rats antagonize the inhibition of acetylcholine release by morphine in vivo (171, 172). Jhamandas et al (173) obtained similar results after i.c.v. injection of Ca^{2+}.

CONCLUSION

In the preceding sections a considerable amount of evidence has been presented which indicates that the actions of narcotic drugs are intimately involved with changes in cellular movement and content of divalent cations and especially Ca^{2+}. At this stage it is worth pointing out that Ca^{2+} or other ions may not be directly involved in the actions of opiates, but that the changes observed may be secondary to drug-induced effects at other unknown loci. Thus, for instance, if morphine acted by inhibiting neurotransmitter release without directly affecting Ca^{2+}, changes in Ca^{2+} flux and/or content might well be observed as a result of the decreased neuronal activity. The reversal of opiate effects by administration of divalent cations both in vivo and in vitro, however, makes this idea less tenable since this better fits a concept of direct interaction between opiates and divalent metal ions.

If it is assumed that narcotic drugs do exert their effects by directly altering the metabolism of Ca^{2+} or other cations, then an attractive hypothesis can be formulated which neatly fits in with known facts. Thus acute narcotic administration produces decreased Ca^{2+} flux and/or binding at the synapse, resulting in reduced neurotransmitter release (thus producing analgesia and other drug effects) and also causing selective decreases in Ca^{2+} levels. A homeostatic mechanism then comes into effect which tends to reverse the effects of the drug resulting in an increased Ca^{2+} content of synaptic vesicles and SPM. Since techniques that elevate intracellular Ca^{2+} necessitate more opiate to produce a response, this adaptation results in tolerance development. Subsequent administration of the higher doses of narcotics now needed to produce an effect in turn gives rise to a greater

adaptive response and more cellular Ca^{2+} accumulates. In this way the level of tolerance increases with increasing doses of the drug. When the constraint exercised by the narcotic drug is removed from tolerant animals, either by naloxone administration or by drug discontinuance, the elevated levels of Ca^{2+} would be free to facilitate a greatly increased degree of neurotransmitter release and thus result in the manifestation of withdrawal hyperactivity.

This model assumes that Ca^{2+} is essential in pain perception and that decreased Ca^{2+} would result in analgesia. In support of this, previously discussed data (34, 41–43, 101) has demonstrated that La^{3+}, EDTA, and EGTA, agents presumed to prevent Ca^{2+} influx into cells, can themselves produce analgesia. Conversely, i.c.v. injection of Ca^{2+}, Mg^{2+}, and Mn^{2+} has been shown to result in hyperalgesia (101). Narcotic tolerance-dependence is taken to be an adaptation to the reduced Ca^{2+} levels caused by acute drug treatment. In agreement with this, evidence has been cited (66–69) which shows that Ca^{2+} administration reduced tolerance development (presumably by overcoming the acute drug effects on Ca^{2+} levels) while EGTA infusion results in increased tolerance development (presumably by enhancing the acute Ca^{2+}-depleting effects of the drug). Tolerance development is accompanied by the development of cross-tolerance to La^{3+} (41, 42) and this again is consistent with the idea that increased brain Ca^{2+} levels can be directly associated with the tolerant-dependent state. Furthermore, the increased levels of Ca^{2+} seen in the tolerant-dependent state should give rise to pronounced hyperalgesia once the narcotic drugs have been withdrawn, while the Ca^{2+}- antagonist properties of La^{3+} should reduce withdrawal symptoms. Again, evidence has been cited that indicates that this is the case (42, 101).

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