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COMMENTARY

BRAIN CALCIUM AND MORPHINE ACTION*

INDRAVADAN S. SANGHVI and SAMUEL GERSHON

Biological Research and Development, USV Pharmaceutical Corp., Tuckahoe, NY 10707 (I. S. S.) and Neuropsychopharmacology Research Unit, Department of Psychiatry, New York University Medical Center, New York, NY 10016 (S. G.), U.S.A.

The requirement for calcium in membrane stabilization and the functioning of neuronal systems is well established [1, 2], particularly in excitation coupling mechanisms [3, 4] and in the regulation of hormone–receptor interactions [5], but the role of calcium in the mechanism of action of opiates has been investigated only recently.

Morphine and calcium

Although it was shown as long ago as the late 1930s and early 1940s that a high calcium diet prevented or delayed the development of tolerance and dependence to morphine in guinea pigs and rats [6, 7], no further studies on the effect of calcium on opiate action were reported until 1966. Intracisternal or subcutaneous administration of calcium prevented the analgesic effect of morphine and meperidine in mice and guinea pigs respectively [8, 9]. However, the action of opiates *in vivo* on calcium metabolism in the nervous system was not investigated until recently, except for one report which showed that nonpharmacological doses of morphine caused a slight decrease in whole brain calcium levels [10].

The acute administration of morphine produced a decrease of tissue calcium in discrete regions of rat brain [11]. Moreover, this decrease was linear, dose dependent, time dependent and occurred to an equal degree in eight discrete regions of the brain. This morphine action was antagonized by naloxone and was stereospecific. Harris *et al.* [12] have demonstrated that prior administration of calcium (as well as magnesium or manganese) to mice prevented the analgesic effect of a single dose of morphine and also that calcium was a more effective antagonist in morphine-tolerant mice than in nontolerant animals. These reports prompted us to examine the effect of chronic morphine on brain calcium levels and the antagonism of concurrent administration of calcium on the naloxone-induced abstinence syndrome in morphine-dependent rats [13]. As in acute experiments, morphine decreased calcium levels in the brain of morphine-dependent animals. Concurrent administration of calcium not only prevented the lowering of calcium but significantly blocked the precipitated abstinence syndrome. Earlier, Kakunaga and Kaneto [14] had shown that repeated intracisternal injections of morphine plus calcium in mice caused less tolerance to the analgesic effect of morphine than was shown in the group of animals receiving morphine alone. Coadministration of naloxone also blocked the development of dependence to morphine [15]. Takemori [16] has defined the agents which modify the development of dependence or withdrawal signs. According to his criteria, when a compound is administered before exposure to a narcotic drug and continued throughout the course of chronic narcotic drug treatment, it should modify the development of dependence. Thus, the ability of concurrent administration of calcium to prevent the withdrawal signs in morphine-dependent rats would suggest that calcium prevented or markedly reduced the development of dependence in rats.

The mechanism of action of calcium in this interaction with morphine is not quite known. The functional role of calcium in the release of putative neurotransmitters is well established. Morphine inhibits the release of ACh† from brain cortical slices *in vitro* [17, 18]. The metabolism of biogenic amines also is affected by morphine (for review, see Ref. 16). However, putative neurotransmitters do not appear to be involved directly in the action of calcium in morphine-treated animals. The evidence in support of such a contention is that magnesium, which has effects opposite to calcium on the transmitter release, also antagonized the analgesic action of morphine [12]. Furthermore, these investigators observed that calcium administration did not affect the ability of agents which alter brain monoamine levels to influence morphine action. Thus, the ability of a monoamine oxidase inhibitor, pargyline, to potentiate the analgesic effect of morphine or the ability of 6-hydroxydopamine (6-OHDA), a catecholamine depletor, to antagonize the effects of morphine were not affected by calcium pre-treatment. An alteration of release of amines by calcium would have changed the relative potency of pargyline and 6-OHDA in potentiating or antagonizing the action of morphine. In fact, Harris *et al.* [12, 19] have suggested that the probable site of calcium action in opiate-treated animals is at the post-synaptic neuronal membrane. Ionophore X537A, which potentiates the effect of calcium, is shown to increase the permeability of the cell membrane to divalent cations [12], indicating that the antagonistic effect of calcium is dependent upon the ion being able to penetrate cell membrane. Considerable experimental evidence indicates that morphine causes changes in the structural environ-

* Literature surveyed to August 1976.

† Abbreviations: Ach, acetylcholine; c-AMP, cyclic adenosine 3′:5′-monophosphate; c-GMP, cyclic guanosine 3′:5′-monophosphate; CDR, calcium-dependent regulator; Ca^{2+}, calcium ion; and PGE_1, prostaglandin E_1.

ment of the cellular membrane and binding and/or the transport of cations across the cell membrane.

It has been suggested that the analgesic effect of morphine may be associated with an inhibitory action of morphine on calcium and magnesium fluxes at the cell membrane in the central nervous system [8, 20]. Calcium and magnesium are bound to the cell membrane and the displacement of calcium or magnesium by narcotics may induce subtle structural changes in cell membranes that affect the transport of these ions [20]. Morphine alters the binding of calcium to neuronal phospholipids and ganglioside [21–23] and to synaptosomal plasma membranes [19]. In fact, morphine is shown to inhibit the uptake of radioactive calcium in brain tissues [17, 24]. Conversely, the addition of physiological concentrations of calcium reduced the binding of opiate ligand to isolated membrane fractions [25–27]. Finally, it has been suggested that changes in calcium localization on the cellular membrane produced by morphine may be involved in the analgesic effect and the development of tolerance and dependence [28], as lanthanum, a trivalent cation, antagonized the effects of acute as well as chronic effects of morphine and also inhibited the binding and movement of calcium across the cell membrane.

Calcium, cyclic nucleotides and morphine

Cyclic-AMP has been established as an intracellular mediator for a large number of substances including hormones and putative neurotransmitters in several tissues including brain [see Ref. 29]. Formation of c-AMP is catalyzed by membranal adenylate cyclase from ATP following stimulation of the post-synaptic membrane by hormones, neurotransmitters or electrical stimulation. Concurrently with this process, an increase in intracellular calcium concentration occurs, most likely by increased uptake of calcium from the external medium [30, 31] or by release of bound intracellular calcium or by both mechanisms [32]. The increased intracellular calcium then enhances the activation of cyclic nucleotide phosphodiesterase (PDE) by its modulator protein which hydrolyzes increased c-AMP at a faster rate [32]. Alternatively, as has been proposed [33, 34], calcium influx results in the formation of a calcium–CDR (calcium-dependent regulator) complex. This Ca^{2+}–CDR complex or protein activator [35] activates adenylate cyclase and a consequent increase in c-AMP occurs; concomitant activation of PDE by a Ca^{2+}–CDR complex would cause a decrease in c-AMP and possibly c-GMP. These observations need further confirmation; nonetheless, there appears to be a self-regulatory system whereby c-AMP stimulates its own breakdown in nerve cells by regulating the activity of its degrading enzyme. In this way an "overstimulation" of the receptors may be avoided. The importance of calcium for the activation of adenylate cyclase is shown by other studies also. In assays *in vitro* in various tissues, a low concentration of calcium (and magnesium) was found necessary for the activation of the enzyme and the formation of c-AMP [36, 37], whereas high concentrations (> 0.5 mM) inhibited the enzyme and the formation of c-AMP [36–40].

The possibility that the cyclic nucleotide system is involved in the action of opiates is documented in several studies. Naito and Kuriyama [41] found that chronic administration of morphine to mice resulted in an increase in adenylate cyclase activity whereas acute morphine had no effect on enzyme activity, but Puri *et al.* [42] reported that both acute and chronic morphine treatment caused an increase in adenylate cyclase activity of rat brain tissue. Collier and Ray [43] suggested that acute and chronic morphine administration inhibited the PGE_1-stimulated adenylate cyclase, and Iwatsubo and Clouet [44] showed an increase in both basal as well as dopamine (DA)-sensitive adenylate cyclase activity after acute morphine treatment of rats, whereas in chronically morphine-treated rats only the DA-sensitive cyclase activity was increased. Finally, in a study, *in vitro*, morphine was shown to inhibit basal as well as PGE_1-stimulated adenylate cyclase of neuroblastoma glioma hybrid cells [45, 46].

Based on the studies cited above, it appears that chronic treatment with opiates results in an initial inhibition followed by a moderate increase in the activity of adenylate cyclase. Sharma *et al.* [45] proposed an hypothesis concerning molecular basis for narcotic tolerance and dependence. They proposed that inhibition of adenylate cyclase by morphine reduces intracellular levels of c-AMP. This may lead to a compensatory shift in enzyme synthesis, degradation or activity which restores the normal level of c-AMP. The cell, then, is dependent upon the narcotic because the level of c-AMP is normal in the presence of the drug and abnormally high upon withdrawal. During the development of dependence, the number of adenylate cyclase molecules increases, which leads to tolerance, since, at a given narcotic concentration, the amount of uninhibited enzyme is greater in dependent than in normal cells. Upon withdrawal, an abnormal rise occurs in the level of c-AMP.

The review of the literature presented so far suggests that chronic morphine treatment affects the metabolism of calcium and cyclic nucleotides. It is tempting to ascribe causal relationship to these observations. Morphine may inhibit adenylate cyclase activity either independent of its action on the transport of calcium or directly by reduced formation of the Ca^{2+}–CDR complex which, as suggested by some investigators, may be essential for the activation of adenylate cyclase [33, 34]. Thus, the following chain of events at the post-synaptic membrane is proposed as one possible mechanism of development of tolerance and dependence to opiates and the consequent withdrawal syndrome. In a normal or non-opiate state, stimulation of the post-synaptic membrane causes an influx of calcium or release of bound calcium from the membrane (in the presence of external calcium). The calcium, in turn, stimulates a protein activator which will activate an "inactive" adenylate cyclase, and an increase in c-AMP will occur. This event is followed by increased levels of calcium in the cytosol which will activate soluble PDE which hydrolyses the c-AMP and re-establishes the homeostasis [34]. During chronic morphine treatment, decreased calcium transport may cause less activation of protein activator and reduced activation of adenylate cyclase and reduced formation of c-AMP. During

development of tolerance and dependence, enzyme induction occurs and, at a given concentration of opiate, more molecules of adenylate cyclase are available for activation. On withdrawal, calcium influx will relatively increase, and active protein activator will be formed. The latter will now activate more molecules of adenylate cyclase and an abnormal rise in c-AMP will ensue associated with the withdrawal syndrome. Such a mechanism may partly explain the significant blockade of precipitated abstinence syndrome when calcium was administered concurrently with morphine [13]. The presence of a high concentration of calcium along with morphine may permit enough calcium influx to activate the protein activator to maintain normal enzyme activity, obviating the necessity for the induction of new enzyme, thus preventing the development of tolerance and dependence and consequent abstinence syndrome following withdrawal. This proposal remains speculative, and further experimental evidence is needed to verify it.

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