REVIEW ARTICLE

IN Volvement of Cyclic AMP and Prostaglandins in Morphine Analgesia, Tolerance and Physical Dependence

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INTRODUCTION

In the last few years, a good number of publications have dealt with biochemical processes that may be involved in either the analgesic effect or in the development of tolerance to and physical dependence on morphine (9, 12, 22, 28, 38). Such studies undoubtedly support the interpretation that a profound alteration of human behavior caused by the chronic use of the opiates should originate in some important biochemical mechanisms within the body.

The consideration of the involvement of cyclic 3'-5' adenosine monophosphate (cyclic AMP; cAMP) and prostaglandins in morphine analgesia, tolerance and physical dependence has emerged from several observations. Prostaglandins are distributed in the brain and other tissues and are very active in a number of biological systems (20). The release of prostaglandins from different areas of the brain has been shown to be induced by several external stimuli (33). Prostaglandins of the E series (PGE) have been shown to cause sedation, stupor and catatonia when administered intraventricularly to cats (21) and produce sedation in rats after an intraperitoneal injection (15). Also, administration of PGE produced emesis, hyperthermia, and hyperglycemia, all effects that are also elicited by morphine and apomorphine (7). A relationship has already been suggested between PGE and the mechanism of analgesia produced by aspirin like analgesics (41). cAMP has repeatedly been implicated as a second messenger which mediates the pharmacological effects of numerous hormones in many tissues (14). Although no definitive role for cAMP in brain function has been assigned, several psychopharmacological agents such as phenothiazine tranquilizers, tricyclic antidepressants, hypnotics, and sedatives have been shown to influence the cAMP content of the brain by affecting either the adenylyl cyclase (AC) or phosphodiesterase (PDE) systems (27).

An attempt has been made in this review to summarize various experimental results and hypotheses presented for the role of cAMP and PGE in morphine analgesia, tolerance and physical dependence. For convenience, the discussion will be divided into four sections: 1) effects of morphine on cAMP levels, 2) effects of morphine on PGE-stimulated cAMP formation,
3) Effects of cAMP on morphine analgesia, tolerance and physical dependence, and 4) effects of PGE on morphine analgesia. A discussion integrating interactions between morphine, cAMP and PGE follows at the end.

Effects of morphine on cAMP levels:

The influence of the opiate on cAMP levels has been studied in various areas of the brain under several experimental conditions. The effects of acute and chronic morphine administration on cAMP levels have also been studied. These effects have been studied in relation to the activities of both enzymes concerned with the regulation of cAMP levels in the cell - AC and PDE (for references, see 42). Unfortunately, the literature contains contradictory data and no uniform picture emerges. For example, acute morphine injection increased the activity of AC in mouse cerebral cortex, had no effect on cerebellum and hypothalamus, and decreased the enzyme activity in the brain stem. Chronic morphine administration decreased AC activity in cerebral cortex of mouse but did not affect the AC obtained from cerebral cortex of rat. However, in another study, chronic administration of morphine induced an increase in AC activity in mouse cerebral cortex as opposed to acute administration which seemed to have no effect. In another report, an injection of morphine to rats caused depletion of cAMP in the hypothalamus and substantia nigra. Naloxone when injected 20 min prior to morphine, prevented the decrease in cAMP by morphine. When the rats were made tolerant by morphine pellet implantation for about 72 hr, there was an increase in cAMP levels in substantia nigra and thalamus but not in the hypothalamus. Injection of naloxone in tolerant animals produced an early elevation of cAMP in the substantia nigra and a decrease in cAMP in the hypothalamus. In some in vitro studies, opposing effects of morphine on AC have been observed. In rat striatum, basal AC was stimulated or was not affected. In other areas of the brain, AC was inhibited. Withdrawal of addicted mice from the narcotic decreased AC activity of cerebral cortex, cerebellum and hypothalamus. Morphine, both in vitro and by acute injection, inhibited high Km PDE of rat striatum but had no effect on high Km PDE in striatum of dependent rats. Van Inwegen et al. also reported nonconsistent effects of morphine in vitro or in vivo on either NaF stimulated or dopamine (DA) stimulated AC of striata of naive, addicted and addicted rats in withdrawal. In their study, PDE in striata from all these treatments showed similar kinetics. Merali and co-workers demonstrated that development of morphine dependence was accompanied by enhanced cAMP metabolism in the striatum as well as in crude synaptosomal fraction of the whole brain and the activity of DA sensitive AC was virtually blocked during withdrawal. Naloxone administration suppressed the rise in cAMP and reversed the morphine stimulated increase in the activities of AC and protein kinase. It was concluded that morphine dependence might be associated with an enhanced metabolism of both, DA and cAMP in the rat striatum and blockade of DA sensitive AC may be related to the withdrawal symptoms.

The contradictions in the various data seem difficult to resolve and to some authors it simply reflects the complexity of the brain and our inability to control the many factors which
are known to affect AC and PDE activities in this tissue. For example, different routes of injection of the opiate, the method used for producing tolerance and dependence (daily injections versus sustained release pellets), the method employed to measure the nucleotide levels or the enzyme activities - these may contribute to the discrepancies seen in the results. The effects of acute morphine administration on cAMP may possibly involve the depletion of catecholamines from the brain structures or alternatively be due to an early release of calcium that may result in a rapid activation of PDE activity (29). There are various studies (for references, see 29) indicating that some of the effects of the narcotics including the development of tolerance and physical dependence can be modified by manipulations of brain amine levels and administration of the opiates has been shown to alter the metabolism of brain biogenic amines.

In summary, it can be said with some certainty that at least PDE does not seem to play an important role in the regulation of the effects of morphine on cAMP levels in the brain tissue. In the case of AC, no consistency in the effects has been observed in the basal, NaF stimulated or DA stimulated AC in different areas of the brain with different schedules of morphine treatment. Some investigators (31) believe that the observed increase in AC activity may be an important factor involved in the development of drug dependence since chronic ethanol consumption, a dependence producing agent, has also been shown to produce a similar increase in the AC activity in the brain (23). However, the decrease in the levels of cAMP as a major consequence of the action of the narcotics has been drawing more experimental attention. There seems to be a reasonable consistency in the reported effects of morphine on the PGE stimulated cAMP accumulation. Since this action of morphine has been implicated to be responsible for its pharmacological effects, it will be dealt with in detail below.

**Effects of morphine on PGE-stimulated cAMP formation:**

Collier and Roy (9) were first to demonstrate that the addition of morphine to homogenate of whole brain of rat inhibited the stimulation of cAMP formation by PGE_1_. Morphine, however, did not affect the basal formation of the cyclic nucleotide and thus, the action seemed to be specific. In another study (10) they determined the specificity of this effect and absolute as well as relative potencies of several opioids, such as levorphanol, heroin, morphine, and methadone. They compared the concentrations of the opiates required to inhibit by 50% the stimulation by PGE_1_ and PGE_2_ of cAMP formation in rat brain homogenate. They also determined the concentrations of morphine achieved in rat brain after a single injection of morphine. A dose of 5 mg/kg sc gave hypothalamic concentration of 0.58 µg/g of the tissue and this was found equivalent to an inhibitory concentration (25%) of morphine against PGE_1_ stimulation of cAMP formation *in vitro* (0.51 µg/ml). They compared the potencies relative to morphine on a molar basis in the PGE-cAMP test with those found in some other tests that may correlate well with the analgesic activity at the site of action. The comparison revealed that the potency of other opiates relative to morphine in PGE-cAMP test fell in between that for affinity to the opiate receptor and that for oral antinociceptive activity. Thus the rank order of potencies of several morphine-like...
drugs in this biochemical test system correlated well with their analgesic potencies. Comparison of the effect of morphine on various AC activities - basal, NaF stimulated and \( \text{PGE}_1 \) stimulated revealed that at concentrations effective against \( \text{PGE}_1 \) stimulated cAMP formation, morphine did not substantially inhibit basal AC activity in rat whole brain homogenates. The morphine antagonist, naloxone, antagonised this action of morphine. In another study, Roy and Collier (34) reported an experiment in which they determined, in the same preparation of rat brain homogenate, the inhibitory effect of various concentrations of morphine on two types of cAMP formation - basal and \( \text{PGE}_1 \) stimulated. A dose-response relationship for the effect of morphine on \( \text{PGE}_1 \) stimulated cAMP levels could be established whereas none of the concentrations of morphine tested had any significant effect on basal cAMP formation. In cultured cells, evidence for stereospecificity is quite convincing. In neuroblastoma cells the effectiveness of morphine was much greater in cells rich in opiate receptors (36). Also, the drug concentration needed for the inhibition of AC by the opiate drugs, with or without \( \text{PGE} \) stimulation, correlated well with the potencies of the same drugs in displacing labelled naloxone from receptor binding sites in these cells. In another report, the same group (25) suggested that the opiate receptors may act as regulators of adenylate cyclase in morphine sensitive cells. They have been able to explain the dualism in agonistic-antagonistic behaviour of nalorphine in the context of opiate action as an inhibitor of AC system. Nalorphine partially inhibits the enzyme in the absence of morphine and in the presence of morphine, nalorphine tends to reverse the inhibition produced by morphine (25). Collier and Roy (10) proposed a hypothesis stating that the analgesic and allied effects of the opiates are caused by inhibition of adenylate cyclase of morphine sensitive neurons which normally responds to prostaglandins of the E series. This hypothesis has been supported by experimental evidence from different laboratories (6, 25, 34, 36, 42). The evidence for this effect of morphine is more conspicuous in cells or tissues which are rich in opiate receptors. However, testing the cerebellum, in which opiate receptors are thought to be absent, has not been done and should be attempted. One study (40) showed that the interaction between morphine and \( \text{PGE}_1 \) was non-competitive. Even in the hybrid cells that respond to morphine, the drug also depressed basal AC activity in the absence of added \( \text{PGE}_1 \). These results also cast some doubt on the role of \( \text{PGE}_1 \). It has been pointed out earlier that in some cases, basal activity responded to the same extent as did \( \text{PGE}_1 \) stimulated activity. It has been observed that morphine stimulated prostaglandin synthesis in bull seminal vesicles as well as rat brain, and this effect was not blocked by naloxone (7, 8). In this respect, the effect seemed unrelated to the interaction with opiate receptors. The stimulation of prostaglandin synthesis may just reflect the possibility of their involvement in the excitatory actions of morphine on the nervous system. More studies concerning the effects of morphine on prostaglandins synthesis are needed. If the PGE-induced cAMP formation is to be implicated in morphine analgesia and dependence then it would be desirable to demonstrate any changes in PGE synthesis or degradation, especially in the brain, that may be associated with the chronic morphinization. It needs to be established if, during dependence development, the sensitivity of \( \text{PGE-cAMP} \) system changes towards morphine or it is the change in the PGE synthesis that is reflected as the changes in cAMP levels. Further discussion of this
hypothesis follows at the end.

Effects of cAMP on morphine analgesia, tolerance and physical dependence:

In the first report (26) of direct antagonism by cAMP of morphine analgesia, it was shown that in mice as well as rats pretreated with 10 mg/kg (iv) of either cAMP or dibutyryl-cAMP, the median analgetic dose (AD50) of morphine was significantly increased at various time intervals. Similar effect was observed with intracerebral injection of 28 μg of cAMP. One report (30) indicated increased activity of cAMP dependent protein kinase in mammalian brain tissue during morphine withdrawal. Later studies (17, 18) have confirmed the antagonistic effects of cAMP on morphine analgesia. The intracerebral cAMP pretreatment afforded antagonism to morphine analgesia for about 35 hr whereas with iv administration the antagonism, as tested by the tail flick method, lasted at least 24 hr. Similar results have been reported when different test methods for analgesia were used e.g. hot-plate method and stretching test (13). Other cyclic nucleotides such as cGMP, cUMP, and cCMP failed to produce any antagonistic effects (18). The results obtained with dibutyryl cAMP were identical to those obtained with iv cAMP in onset, degree and duration. Both, cAMP as well as dibutyryl cAMP were efficient antagonist in nontolerant and tolerant mice (17). In a recent study (19), a single iv injection of cAMP markedly accelerated tolerance development in mice. Based on the relative increase in their respective AD 50 values, the cAMP group was more than 3 times as tolerant as the control group. Similar experiment with cAMP resulted in a doubling of the morphine AD 50 over that of saline-treated mice. The injection of cAMP prior to morphine pellet implantation also accelerated the development of physical dependence which was indicated by a decrease in the amount of naloxone needed to induce precipitated withdrawal jumping. In these studies (18), neither 2’3’ cAMP or cGMP had any effect on the development of tolerance and dependence. Other indirect methods have been used to characterize this effect. Theophylline, an inhibitor of cAMP phosphodiesterase, produced a degree of antagonism that was roughly comparable to that obtained with cAMP (17). Theophylline, when administered 2 hr before morphine pellet implantation and then given every 24 hr for 2 additional days was also found to enhance tolerance and physical dependence development (19). Theophylline produced a “quasi-morphine abstinence syndrome” that was intensified by naloxone and suppressed by heroin (5). Collier and Francis (4) showed that the expression of abstinence was associated with an increase in the brain cAMP. Various PDE inhibitors (theophylline, caffeine, 3-isobutyl-1-methylxanthine) given subcutaneously to the dependent rats one hr before challenge increased the occurrence of jumping and several other withdrawal signs. In the same study, imidazole, which stimulates PDE, had an opposing effect. There are several points to be noted, however. Gourley and Bakner (13) have shown that this type of antagonism is not specific for cAMP. Adenine and adenosine when given before the analgesia test were at least as effective as cAMP in the analgesic tests. AMP and ATP were also quite effective in antagonizing morphine analgesia whereas ADP was not. The requirement of an intact adenine structure for an effective antagonism was also suggested by them. These studies
dealt only with morphine analgesia and should be carried out in cases of tolerance and dependence in order to assess the specificity of the action of cAMP. Of course, the above results do not exclude the possibility that exogenous adenine, adenosine, and adenine nucleotides resulted in an increase in the synthesis of cAMP in the CNS; such a possibility would be of interest to verify. Another point is that PDE inhibitors are known to exert many actions unrelated to the enzyme inhibition (2) and thus the results with these agents should be treated with some reservation. The same is felt for dibutyryl cAMP since it is thought that the metabolic and physiological effects of cAMP and dibutyryl cAMP may differ (16).

Effects of prostaglandins on morphine analgesia:

Studies on the effects of prostaglandins on morphine analgesia are limited in number. Ferri et al. (11) demonstrated that intraventricular administration of PGE₁ in rats 60 min after morphine injection induced a significant reduction of the threshold to the pain stimulus. The PGE₁ effect reached its maximum 10 min after administration and then the analgesic threshold gradually approached the level of the animals treated with morphine alone. One possible explanation for the antagonistic effect of PGE₁ is an increase in cAMP produced by PGE₁. However, a more detailed study utilizing different dosage schedules for PGE₁ as well as morphine is warranted for any conclusive interpretation. In this experiment, PGE₁ was given after the morphine administration. Antagonism by PGE₁ should also be shown by the administration of PGE₁ prior to morphine. When PGE₁ was given to rats 30 min before morphine, it significantly potentiated the antinociceptive action of a subanalgetic dose of morphine. Also, the effects of prostaglandins on morphine tolerance and physical dependence have not been studied.

CONCLUSION

If the interrelationship of morphine, prostaglandins and cAMP is to be observed in the context of the above experimental evidence, it seems that the majority of data, while not absolutely conclusive, favor the hypothesis proposed by Collier and Roy (10), that a major biochemical action of morphine is to cause the inhibition of PGE₁ stimulated cAMP in the neurons that are rich in opiate receptor content. The binding of the opiate molecule with its receptor may produce a conformational change in the catalytic site of adenylate cyclase that reduces the enzyme’s capacity to convert ATP to cAMP, whereas binding of PGE to a different unrelated site would enhance the AC activity. The opiate dependence and tolerance is then explained by saying that after chronic administration of morphine there is a biochemical hypertrophy that would compensate for the inhibition (6, 25). Thus, on continued exposure to morphine the cells can adapt by an increase in AC activity which results in tolerance and dependence because now more morphine would be required to cause the same degree of AC inhibition. The fully tolerant cells may have cAMP levels close to normal in the presence of morphine. When the opiate is withdrawn or on addition of a narcotic antagonist, cAMP level rise to abnormally high values because of the removal of the inhibitory regulator. This abrupt increase in cAMP is the biochemical counterpart of the abstinence syndrome. Recovery of the cells from the addicted state requires the
return of AC activity to its normal levels. An important feature of this postulate is the suggestion that cAMP has the primary role. The studies dealing with the role of calcium in the mechanism of action of opiates suggest that there may be a link between morphine, cAMP and Ca$^{2+}$ (for references, see 35). Chronic morphine treatment affects the metabolism of calcium and cyclic nucleotides and thus a causal relationship between these observations has been ascribed (35). However, the proposal remains speculative, and further experimentation is needed to verify it.

REFERENCES


