Consequences of Interaction between Opioid and Receptor

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Gaddum (1962) divided receptors into 'pharmacological' and others. Pharmacological receptors give a pharmacological response to binding of molecules of drug (including endogenous substances) and are blocked by specific antagonists. One such is the opiate receptor. Its ligands include opiates (derivatives or opium), opioid drugs (e.g. methadone, pethidine), opioid peptides (endorphins, including enkephalins), opioid antagonists (e.g. naloxone, naltrexone) and partial antagonists (e.g. cyclazocine, nalorphine).

The 'morphine-sensitive neuron' is one possessing opiate receptors and giving a characteristic pharmacological response to the binding of opioid agonist molecules with these receptors. The pharmacological consequences of agonist binding are of two types, namely acute and sub-acute. The acute agonist actions of opioids include analgesia, respiratory depression and inhibition of the electrically evoked contraction of the isolated guinea-pig ileum. The sub-acute agonist actions are the induction of tolerance and dependence. The binding of antagonist blocks both acute and sub-acute opioid actions.

The present paper asks 'what events leading to the pharmacological response occur in the morphine-sensitive neuron after binding of opioid agonist molecules with their receptors?' The duality of opioid action, however, means that this question is triple: (1) 'What event leads to the acute agonist action of opioids?' (2) 'What event leads to the induction of tolerance and dependence?' (3) 'At what point in the sequence of events do the paths leading to the acute and sub-acute actions divide?'

Four arguments led us to explore the interaction of opioids with the cyclic AMP system of nervous tissue as the event after drug-receptor binding that generated their agonist actions. First, although morphine does not inhibit prostaglandin biosynthesis, E prostaglandins elicit or enhance several of the reactions, such as pain, cough, diarrhoea and fever, that morphine suppresses (Collier & Roy, 1974a), and, in some instances (e.g. diarrhoea), E prostaglandins act by stimulating adenylate cyclase. Secondly, cyclic AMP and the phosphodiesterase inhibitor, theophylline, antagonize the anti-nociceptive action of morphine (Ho et al., 1973). Thirdly, morphine interacts with such a variety of humoral transmitter substances that its primary interaction might well be with a mechanism affecting many transmitters (Collier, 1973). Fourthly, since it was unlikely that mammals had evolved with appreciable amounts of opiates in their tissues, the opiate receptor was likely to be the receptor for an unidentified endogenous substance (Collier, 1973). If this substance acted as a central transmitter or modulator, it might be expected to interact directly with a cyclic nucleotide mechanism.

We approached this analysis of opioid agonist actions from both ends. At one end, we tested whether morphine inhibited basal or prostaglandin E-stimulated cyclic AMP formation in homogenates of rat intestine and brain. At the other end, we tested whether a phosphodiesterase inhibitor would produce in drug-naive rats behaviour comparable with the morphine-abstinence syndrome. Since both approaches yielded positive and mutually consistent results, we also explored what happened in between.

Acute agonist actions

In rat brain homogenate, opiates inhibited prostaglandin E-stimulated, but not basal nor fluoride-stimulated, adenylate cyclase (Collier & Roy, 1974a,b). The inhibitory effect of opiates was dose-related and, in a series of opiates, its potency was correlated with analgesic activity. Inhibition occurred at concentrations reasonably close to those in brain at analgesic doses in vivo. The inhibition of adenylate cyclase by opiates was antagonized by naloxone or naltrexone and was stereospecific. From this and other evidence, we proposed that the inhibition by
opiates of prostaglandin E-stimulated adenylate cyclase of morphine-sensitive neurons represented the biochemical mechanism of the analgesic and allied acute agonist actions of these drugs. This proposition requires that the opioid peptides, discovered subsequently, should also inhibit prostaglandin E-stimulated cyclic AMP formation in rat brain homogenate. This they do; but they also, though with lower potency, inhibit basal cyclic AMP formation (H. O. J. Collier, A. C. Roy & D. G. Smyth, unpublished work).

These findings were extended and largely confirmed in cultured neuroblastoma × glioma cells that possess opiate receptors (Traber et al., 1975a; Sharma et al., 1975a) except that Sharma et al. (1975a) found that opiates inhibit basal and adenosine-stimulated, as well as prostaglandin E-stimulated cyclic AMP formation. The enkephalins also inhibit adenylate cyclase in neuroblastoma × glioma cells (Brandt et al., 1976; Klee et al., 1976). In addition to inhibiting adenylate cyclase, the opioids stimulate cyclic GMP formation in these neuroblastoma × glioma cells (Gullis et al., 1975; Brandt et al., 1976). Comparable results have also been obtained in slices of rat neostriatum (Minneman & Iversen, 1976).

Taken together, these latter observations raise two questions about the proposition that opiates act by inhibiting a prostaglandin E-stimulated adenylase cyclase. First, 'what is the relative importance for agonist actions of raising cyclic GMP and lowering cyclic AMP?' Secondly, 'if decreasing the cyclic AMP concentration is important, what is the role, if any, of prostaglandin E stimulation?' Experiments on isolated guinea-pig ileum have helped to answer these questions.

An acute agonist action of morphine is to inhibit the electrically evoked release of acetylcholine from the postganglionic neurons of the myenteric plexus of the isolated guinea-pig ileum, thus diminishing contraction of the longitudinal muscle. Prostaglandins E1 and E2 reverse the inhibition by morphine of the longitudinal muscle contraction (Ehrenpreis et al., 1973). That this reversal occurs in the presence of enough hexamethonium to block the ganglion and at concentrations of prostaglandin that do not contract the muscle (Hammond et al., 1976), but enhance acetylcholine liberation (Schulz & Herz, 1976), argues that it is located in the morphine-sensitive neurons of the preparation. In the presence of hexamethonium, caffeine and 3-isobutyl-1-methylxanthine also reverse this inhibition. The relative potency of 3-isobutyl-1-methylxanthine to caffeine in reversing morphine inhibition of the ileum is close to that inhibiting cyclic AMP, but not cyclic GMP hydrolysis, in rat brain homogenate (Collier et al., 1977). These observations argue that a decrease in cyclic AMP concentration is more important than an increase in cyclic GMP concentration for the acute agonist actions of morphine, and that the adenylate cyclase inhibited is one responding to E prostaglandins. Although morphine and E prostaglandins do not interact directly at the same receptor, many experiments give evidence of an antagonism between them. For example, intracerebroventricular injection of prostaglandin E1 briefly antagonizes the anti-nociceptive action of morphine in the rat (Ferri et al., 1974).

Mechanism of withdrawal effects

In an attempt to implicate a cyclic AMP system of brain in morphine-dependence, we also tested whether the phosphodiesterase inhibitor, theophylline, would produce in naive rats a behaviour pattern comparable with the morphine-withdrawal syndrome (Collier, 1974; Collier et al., 1974, 1977; Francis et al., 1975). Not only did a pattern of behaviour resembling the withdrawal syndrome result from large doses of theophylline and other methylxanthines, but this could be intensified by a small dose of naloxone and inhibited by opioids. We called this the 'quasi-morphine-withdrawal (or abstinence) syndrome'. Both intensification of the syndrome by opioid antagonists and its suppression by opioids were stereospecific. The potencies of opioids in suppressing the syndrome correlated closely with their potencies in analgesic and allied agonist actions.
The quasi-morphine-withdrawal syndrome might have been caused by increased brain cyclic AMP or cyclic GMP concentrations or both, or by some other effect of theophylline. Subsequent work showed that the syndrome was elicited by all of seven phosphodiesterase inhibitors tested, and that, taking probable activity \textit{in vivo} into account, their effectiveness in eliciting a syndrome correlated with their potency in inhibiting cyclic AMP, but not cyclic GMP, hydrolysis in rat brain homogenate (Collier \textit{et al.}, 1977).

Comparable results were obtained in the true-morphine-withdrawal syndrome of rats (Collier & Francis, 1975). Given shortly before naloxone to dependent rats, phosphodiesterase inhibitors intensified, and the phosphodiesterase stimulant, imidazole, lessened withdrawal effects. Also, when cyclic AMP, cyclic GMP or a dibutylryl derivative was injected into a lateral cerebral ventricle of dependent rats shortly before withdrawal was precipitated with naloxone, cyclic AMP intensified, but cyclic GMP inhibited, some withdrawal effects.

It is consistent with these findings that the total content of brain cyclic AMP during naloxone-precipitated withdrawal increases sharply, to an extent related to the intensity of the withdrawal syndrome (Mehta & Johnson, 1974). Also, exposure of neuroblastoma\texttimes glioma cells in culture for some hours to morphine solution results in a greatly increased production of cyclic AMP on challenge of the cells with naloxone (Sharma \textit{et al.}, 1975b) or with prostaglandin E\textsubscript{1} (Traber \textit{et al.}, 1975b). In opiate-dependence, supersensitivity to prostaglandin E has been observed not only in neuroblastoma\texttimes glioma cells, but also in other preparations (Collier \textit{et al.}, 1975; Weeks & Collins, 1976; Schultz & Herz, 1976).

These observations led us to conclude that morphine-dependence is a state of increased capacity for increasing the concentration of cyclic AMP in appropriate neurons (Collier & Francis, 1975). If so, the question arises 'how does this capacity increase?' Experiments on tolerance in isolated guinea-pig ileum help to answer this.

\textit{Mechanism of induction of tolerance/dependence}

J. E. Villarreal, J. N. Martinez & A. Castro (personal communication) found that dependence could be induced in the ileum \textit{in vitro} by keeping it in morphine solution for 24h at 4°C. After this treatment, but not before it, the ileum responded with a sustained contraction to a small dose of naloxone, indicating dependence. We have confirmed this observation and found that tolerance is also induced in this way (Hammond \textit{et al.}, 1976).

Overnight incubation in morphine (0.35–17.5 \textmu M) in Krebs-Ringer solution at 4°C induced in the ileum both tolerance and dependence. Tolerance was dose-related, and it was not removed by washing the ileum for 45min in three changes of Krebs-Ringer solution. Tolerance persisted for some hours after removing the ileum from the morphine solution that induced it. The tolerance was thus readily measurable, although the response to naloxone was less so. An apparently comparable tolerance could be induced by shorter exposure of the ileum at 37°C.

The tolerance induced in isolated ileum resembled in many ways tolerance induced by morphine in experimental animals. In particular, the concentrations of morphine required \textit{in vitro} and \textit{in vivo} were comparable, and induction was blocked by naloxone (\textit{P}<0.005) or cycloheximide (\textit{P}<0.005).

We therefore tested the effects of drugs on the induction of tolerance by overnight exposure of the ileum to morphine at 4°C. Hexamethonium did not block the induction or the expression of tolerance, indicating that tolerance occurs in the postganglionic neurons of the myenteric plexus, which are morphine-sensitive. By contrast, prostaglandin E\textsubscript{2} inhibited tolerance induction, arguing that inhibition of adenylate cyclase plays a part in this induction.

Unexpectedly, caffeine, which blocked morphine agonist action on the ileum, intensified tolerance induction. This suggested that an increase in cyclic GMP concentration might be important in this process, so we tested the effect on induction of tolerance of exposure to cyclic nucleotide derivatives. 8-Bromo cyclic
GMP intensified induction \((P<0.01)\), but dibutyryl cyclic AMP inhibited it \((P<0.02)\). How an increase in cyclic GMP concentration might intensify tolerance/dependence is unknown, but that cycloheximide blocks tolerance induction in the ileum and that washing does not remove tolerance suggests that formation of a neuron-bound protein is responsible.

In summary, evidence has been presented for the following propositions. Binding of opioid agonist with receptor inhibits an adenylate cyclase of morphine-sensitive neurons, leading to the acute, and contributing to the sub-acute, agonist actions of the drug. An adenylate cyclase that responds to morphine can be stimulated by EP prostaglandins. The opioid-receptor binding also stimulates cyclic GMP formation, which contributes to induction of tolerance/dependence. Tolerance/dependence is a state of increased capacity for increasing cyclic AMP concentration in morphine-sensitive neurons.


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**The Muscarinic Receptor: Isolation and Characterization**

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