OPIATE RECEPTORS AS REGULATORS OF ADENYLATE CYCLASE

Werner A. Klee, Shail K. Sharma* and Marshall Nirenberg
Laboratory of General and Comparative Biochemistry, NIMH, and the Laboratory of Biochemical Genetics, NHLI, NIH, Bethesda, Maryland 20014

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We have reported the presence of opiate receptors in some neuroblastoma derived cell lines cultured in vitro and that a neuroblastoma x glioma hybrid cell line, NG108-15, contains a particularly large number of morphine receptors (1). Table 1 shows that whereas the hybrid cell line has opiate receptors

Table 1 RECEPTOR BINDING

	^{3}H-dihydromorphine		^{3}H naloxone	
	fmoles/mg protein			
HYBRIDS				
NG108-15	17		37	(19)
PARENTS				
N18TG-2	0		11	(6)
C6BU-1	1		1	(1)

The concentration of radioactive narcotic was 1 nM in each case. In neither case is this close to a saturating amount, naloxone one has twice the affinity of dihydromorphine and so to be comparable the naloxone data should be divided by 2 (numbers in parenthesis). NG108-15 (also called 108CC15) was obtained by B. Hamprecht, T. Amano and M. Nirenberg (in preparation), N18TG-2 by Minna et al. (2), C6BU-1 by Amano et al. (3).

which are readily demonstrated by both radioactive ligands those of N18TG2 were only detected with ^{3}H-naloxone binding whereas the C6BU-1 line does not have a detectable number of opiate receptors by either assay method. This group of cell lines with no, few and an abundance of opiate receptors has provided us with material with which to study the biochemical consequences of the interaction of morphine with its receptor.

Collier and Roy reported that morphine and related drugs inhibit the PGE_1 stimulated conversion of ^{3}H ATP into cAMP by rat brain homogenates in a way that correlates with agonist potency and receptor affinity (4,5). These experiments prompted us to examine the effect of morphine on adenylate cyclase activity and on the cAMP levels of NG108-15 hybrid cells and their parental cell lines (6). We found that morphine inhibits the adenylate cyclase activity of NG108-15 cells and lowers cellular cAMP levels in the presence and in the absence of added PGE_1 (fig 1).

*Fogarty International Fellow, on leave from the Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India.

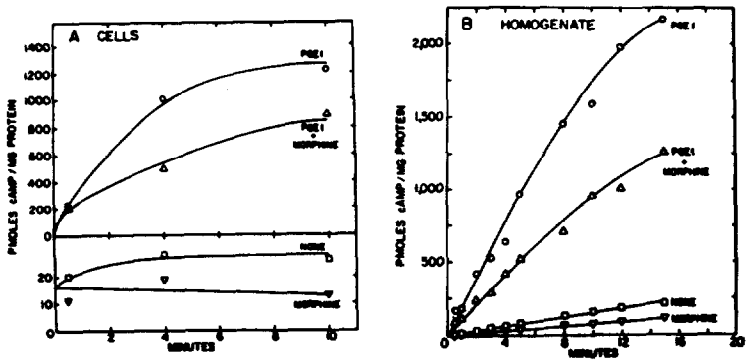

Figure 1. Inhibition by morphine (10^{-5}M) of the rate of cAMP accumulation in intact NG108-15 hybrid cells (part A) and of adenylate cyclase activity in homogenates (part B). Basal and PGE_1 (10^{-5}M) stimulated results are shown (6).

There is also a dramatic reduction in the adenosine stimulated rise in cellular cAMP levels in the presence of morphine (17). Thus, in this cell, morphine inhibits both stimulated and unstimulated adenylate cyclase.

Morphine inhibits the adenylate cyclase of the neuroblastoma parent somewhat, but does not affect the activity the enzyme found in the glioma parent (Table II). Thus, the degree of inhibition of adenylate cyclase by morphine is correlated with the number of opiate receptors. There are a number of other properties of the enzyme of NG108-15 cells which show that the inhibition by opiates is mediated by their receptors. Thus, naloxone, an apparently pure antagonist of narcotic drugs reverses morphine inhibition of adenylate cyclase (6). Furthermore, the inhibition is sterospecific in that levorphanol, but not its inactive isomer, dextrorphan, inhibits adenylate cyclase (6). Traber et al. (7-9) have also reported that morphine reduces PGE elevation of cAMP levels and Blosser et al. (10) have reported that morphine inhibits the PGE_1 dependent activation of adenylate cylase in neuroblastoma or hybrid cell lines derived from neuroblastoma cells.

Table II Effect of morphine on adenylate cyclase activity of neuroblastoma and glioma parents

Addition*	N18TG-2 pmole/min/mg protein	C6BU-1
None	6	20
Morphine	4	19
Naloxone	5	20
Morphine + naloxone	5	20
PGE_1	75	24
PGE_1 + morphine	63	25
PGE_1 + naloxone	72	24
PGE_1 morphine + naloxone	70	23

*10μm of each component

There is a good correlation between the concentrations at which opiate agonists displace ^{3}H-naloxone from the receptors and and those required for inhibition of adenylate cyclase. This agreement is readily apparent in the data presented in Table III.

Table III Comparison of narcotic affinity for the opiate receptor and ability to inhibit adenylate cyclase

Narcotic	Kd Narcotic receptor nM	Ki Adenylate cyclase nM
Etorphine	5	10
Levorphanol	200	200
Morphine	4,000	2,000
3-Allylprodine	10,000	50,000
Dextrorphan	10,000	--
Naloxone	20	--

However, we found that the opiate binding and enzyme inhibition curves not superimposible (6). Enzyme inhibition takes place over a much narrower range of drug concentrations than does displacement of ^{3}H-naloxone from the receptors. This behavior implies cooperativity among liganded receptors in their interaction with the adenylate cyclase complex. Analysis of the data by means of Hill plots shows that the slope for narcotic binding (reaction1 of scheme 1) is close to 1 indicating little or no cooperativity

(1) $M + \text{receptor} \overset{1}{\rightleftharpoons} \text{receptor}\cdot M \overset{2}{\rightleftharpoons} [\text{enzyme}\cdot\text{receptor}\cdot M]$

in the formation of [narcotic•receptor] complex but that the maximum slopes of the curves for adenylate cyclase inhibition by narcotics (reaction 2) are between 2 and 3, indicating strong positive cooperativity in the reactions that couple [narcotic-receptor] complexes with adenylate cyclase.

Coupling of opiate-receptor complexes to adenylate cyclase may occur by any of 3 general mechanisms:

1) Direct interaction of receptor and enzyme, by analogy with enzyme systems composed of catalytic and regulatory subunits, or linkage via a modulator (15).

2) Opiate-receptor complexes may elicit the production of chemical messages, suggested as a rather unlikely possibility by H.O.J. Collier (personal communication).

3) Indirect coupling mediated by conformational transitions of the membrane. The membrane conformation may reflect either the proportions of receptors in states A and B or may change in reponse to the process of transition of the conformation of the receptors between states A and B induced by the association and dissociation of agonists (but not of antagonists, since Na^+ maintains receptors in the B state). The indirect coupling mechanisms, which involve membrane changes, may allow opiate receptors to express their interaction with narcotics in more than one way. Thus, the inhibition of adenylate cyclase and the electrophysiologocal effects of narcotics on NG108-15 cells found by Traber et al. (9), Myers and Livengood (16) and in our own laboratories can be different manifestations of the same fundamental effects on membrane structure.

An important property of many of the narcotic analgesics is that of mixed agonist-antagonist behavior. Perhaps the best studied example of such a compound is nalorphine which is a potent antagonist of morphine, but also is a good analgesic in its own right (11). How may this dualism of action be understood

in the context of opiate action as an inhibitor of adenylate cyclase? Figure 2 shows the effects of nalorphine upon adenylate

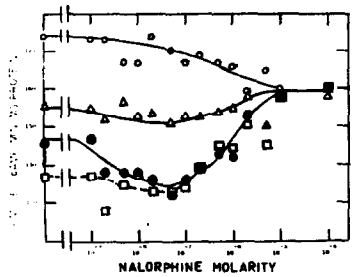

Figure 2. Effects of nalorphine on the adenylate cyclase activity of homogenates of NG108-15 cells. The curves, reading from top to bottom, represent experiments performed at the following concentrations of morphine: none, 2 x 10 M, 10^{-4}M, and 2×10^{-5}M. The data have been normalized so that uninhibited adenylate cyclase activity is constant.

cyclase activity at several concentrations of morphine. In the absence of morphine, nalorphine inhibits the enzyme but only partially when compared with the degree of inhibition produced by morphine. In the presence of morphine, on the other hand, the effect of nalorphine is to reverse the inhibition produced by morphine. The reversal of morphine inhibition by nalorphine is also not complete but only restores enzyme activity to the level seen in the presence of nalorphine alone.

There is evidence that opiate receptors exist in two conformational states (12, 13) as shown below:

$$\text{Receptor A} \underset{}{\overset{Na^+}{\rightleftharpoons}} \text{Receptor B}$$

$$[\text{Agonist} \cdot \text{Receptor A}] \qquad [\text{Antagonist} \cdot \text{Receptor B}]$$

inhibition of adenylate cyclase — uncoupled

Receptor form A has a high affinity for agonists and form B for antagonists as shown. Binding of agonists to the receptor will shift the equilibrium to the left and convert most receptors to the [Agonist•Receptor A] complex which will result in inhibition of adenylate cyclase. Conversely, when receptors are in the form of the [Antagonist•Receptor B] complex, as the result of interaction with a pure antagonist, adenylate cyclase is not inhibited. Mixed agonist-antagonists, such as nalorphine, may have a comparable affinity for receptors in both states. The interaction of opiate receptors with agonist-antagonist narcotics will then result in the receptor complexes being partitioned between states A and B in comparable amounts. Inhibition of adenylate cyclase will thus be only partial as is observed.

When NG108-15 cells are cultured in the presence of morphine for a number of days, the level of adenylate cyclase activity increases by approximately 50-100%. An experiment which demonstrates this phenomenon is shown in fig 3. The cells after 2 or more days of exposure to morphine are tolerant in the sense that adenylate cyclase activity is nearly normal when assayed in the

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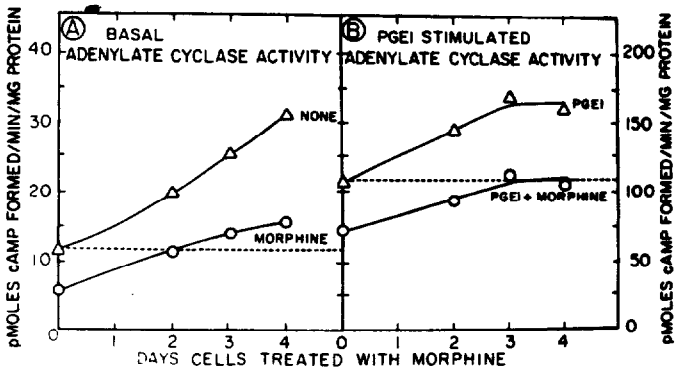

Figure 3. Basal and PGE_1 stimulated adenylate cyclase activity of homogenates of NG108-15 cells cultured in the presence of 10^{-5} M morphine for the times shown (17).

presence of morphine. They are dependent upon it in the sense that adenylate cyclase activity measured in its absence is abnormally high. The dependence phenomenon is dramatically seen when cAMP levels of cells cultured in the presence of morphine for 48 hours are measured after a brief exposure to naloxone.

Table IV cAMP levels of normal and addicted cells (17)

Conditions of Assay	CELLS Normal	Addicted	% of Normal
	pmoles cAMP/mg protein		
basal	20	23	113
naloxone	21	37	175
PGE	264	81	31
PGE + naloxone	241	1183	491
adenosine	103	65	63
adenosine + naloxone	72	217	301
naloxone			

Addicted cells show as much as a 4 to 5 fold increase in cAMP levels over the control, in the presence but not in the absence of naloxone precipitated withdrawal. There is no change in the number of opiate receptors in tolerant cells (17).

Figure 4 summarizes the general conclusions which we have reached concerning tolerance and dependence on the basis of these and other (17), related, experiments. We find that morphine inhibits adenylate cyclase activity and thus decreases cAMP levels. On continued exposure to morphine the cells adapt by an increase in adenylate cyclase activity which results in tolerance and dependence. The fully tolerant cells have cAMP levels close to normal in the presence of morphine. When the opiate is withdrawn on addition of an antagonist, cAMP levels rise to abnormally high values. This abrupt increase in cAMP indicates that the cells are depen-

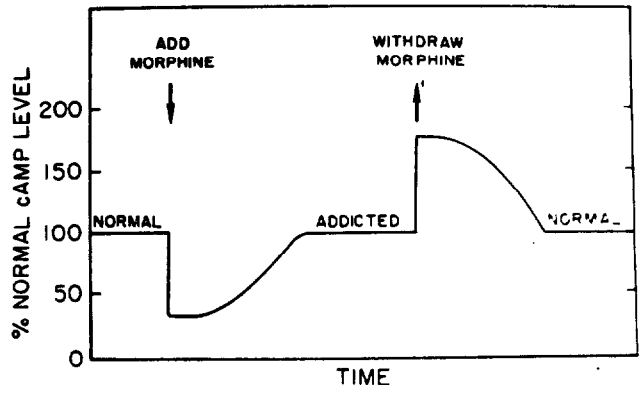

Figure 4.

dent upon morphine and is the biochemical counterpart of the abstinence syndrome. Recovery of the cells from the addicted state requires the return of adenylate cyclase activity to its normal levels. These results support the suggestions of Goldstein and Goldstein (14) and Shuster (19), made many years ago, that drugs may act as enzyme inducers. Increases in cAMP levels in the abstinence syndrome of animals has recently been demonstrated by Collier and Francis (18).

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