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Function of delta opioid receptors in cultured cells

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Summary

The opiate receptor subtype with a high affinity for the endogenous opioids met[5]-enkephalin and leu[5]-enkephalin (δ receptor), has been demonstrated in murine neuroblastoma clones and their glioma hybrids to effect a number of biochemical events, upon agonist binding. These receptors are negatively coupled to adenylate cyclase, and, recent evidence indicates, positively coupled to guanylate cyclase. As has been demonstrated for some other receptors which are negatively coupled to adenylate cyclase, the δ receptor, upon chronic incubation with agonist, increases the basal and receptor-stimulated adenylate cyclase activity compensatory to the inhibition seen with acute exposure (a state of tolerance and dependence); upon removal of agonist (withdrawal), the adenylate cyclase activity returns to normal. The δ receptor is regulated both in activity and number by the processes of desensitization and down-regulation; the maximum number of δ receptor sites is regulated with respect to cellular division. In the same fashion as a number of polypeptide receptors, the δ receptors undergo aggregation into clusters on the cellular membrane upon agonist binding, but, unlike other receptors, these clusters are not subsequently internalized; clustering of the δ receptors is not required for binding of agonist or for inhibition of receptor-activated adenylate cyclase activity. While an increase in the content of unsaturated fatty acids in the cellular membrane (generally resulting in an increase in fluidity of the membrane) results in a decrease in the maximum number of δ binding sites, differing evidence exists as to the effect this change has on the expression of δ receptor function. The integrity of membranous phospholipids is important for the function of the receptor, whereas the incorporation of extraneous cerebroside sulfate into the cellular membrane enhances the activity of the δ receptor in N18TG2 cells but not in N4TG1 cells. Upon chronic exposure to opiates or opioids, N4TG1 neuroblastoma and NG108-15 neuroblastoma x glioma hybrids exhibit a reduction in synthesis of total gangliosides and membranous glycoproteins.

Introduction

Five classes of opiate receptors are believed to exist in the nervous systems of a number of vertebrates (1), based upon binding and pharmacological data: mu (μ), delta (δ), kappa (κ), sigma (θ), and epsilon (ϵ) (see reviews 2–4). These receptors preferentially bind morphine, enkephalins, drugs in the family of ketocyclazocine, drugs related to N-allylnormetazocine (SKF 10,047), and β-endorphin, respectively.

The significance of the existence of different classes of opiate receptors is not completely understood. In particular, the role of the δ receptor, postulated to be of physiological importance subsequent to the early discovery of the endogenous peptides met[5]-enkephalin and leu[5]-enkephalin (5), is not yet elucidated. This is, in part, due to the lack of evidence connecting a distinct biological response to δ receptor activation. While suggested

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Table 1. Delta receptors in neuroblastoma and neuroblastoma x glioma clones.

Clone	Ligand	K_D (nM)	Sites/cell	Ref.
NG108-15[a]	[^{3}H]leu^5-enkephalin	5	–	70
	[^{3}H]leu^5-enkephalin	3.5	410 000	71
	[^{3}H](D-ala^2)-met^5-enkephalinamide	0.6	–	21
	[^{3}H]dihydromorphine	20–30	~300 000	72
N4TG1	[^{125}I](D-ala^2, D-leu^5)-enkephalin	1–2	18 000	73
N1E-115	[^{3}H]met^5-enkephalin	2.4	8 000	23
N18TG2[b]	[^{3}H]naloxone	25	60 000	64
	[^{3}H](D-ala^2)-met^5-enkephalinamide	1.4	–	21

[a] Also has ε receptors (74) at which β-endorphin has a substantially higher affinity than morphine or leu^5-enkephalin.

[b] Also has ε receptors (75) at which neither morphine nor enkephalin inhibit [^{3}H]β-endorphin binding.

functions for the δ receptor *in vivo* range from antinociception to neurotransmitter modulation (see reviews 6–8), definite assignment is complicated by the presence of a non-trivial number of opioids, precursors, and opiate receptor subtypes in the central and peripheral nervous systems.

Investigation of the function and regulation of the δ receptor is immeasurably aided by the utilization of murine neuroblastoma clones from a spontaneous tumor found in the abdominal cavity of a mouse (C-1300) (see Table 1). These clones typically have properties of normal differentiated neurons (9), are homogeneous, are easily obtained in large quantities by cell culture, and bear only one class of opiate receptors, the δ receptor (10). (It should be noted that ε receptors are reported in neuroblastoma clone N18TG2; however, there is no apparent inhibition of β-endorphin binding at these receptors by morphine or enkephalins.) While it is obvious that data obtained on the δ receptor with these cells must be verifiable in the mammalian, and ultimately human, nervous systems to be meaningful, the neuroblastoma clones nevertheless provide a less complex organization for initiation of study.

From investigations with neuroblastoma cells and clones of their glioma hybrids, a number of biochemical events are attributed to the delta receptor upon activation by its natural (met^5-enkephalin and leu^5-enkephalin) and synthetic (enkephalin derivatives) agonists. This review will attempt to summarize what is currently known about these *in vitro* δ receptor-activated effects. The term opiate will be used throughout this paper in reference to drugs isolated from opium (e.g., morphine, codeine, etc.) or to derivatives of morphine, while the word opioid will be employed to designate any compound possessing opiate activity but non-alkaloid structure (e.g., the enkephalin peptides).

I. Delta receptor coupling to adenylate cyclase

Upon binding to agonist, polypeptide-hormone and neurotransmitter receptors on the plasma membrane of target cells initiate a transmembrane series of events thereby triggering specific biological functions. Much remains to be learned about the transfer of information by a hormone or neurotransmitter, e.g., the physical changes in a receptor resulting from activation by binding to a specific agonist, subsequent receptor-initiated membranous reactions, the physical proximity of extra- and intracellular reactants, and the ultimate intracellular actions and biological functions. Some knowledge has been accrued, however, in the area of production of intracellular second messengers following coupling of an activated receptor to the enzymatic effector in the cellular membrane.

General acceptance of cyclic nucleotides as second messengers which amplify and effect some of the intracellular functions of activated neurotransmitter receptors in the mammalian nervous

system began with the discovery in the early 1960s that the central nervous system has a higher content of the enzymes responsible for the synthesis and degradation of cyclic AMP (cyclic adenosine 3', 5'-monophosphate) than does any other tissue (11). The importance of cyclic GMP (cyclic guanosine 3', 5'-monophosphate) as a second messenger which is synthesized and functions in an opposing fashion to cyclic AMP was subsequently postulated (12). Cyclic nucleotides are thought to control or modulate many biological processes, from cellular shape and growth to specific enzyme function (13). However, the primary biochemical reaction known to require cyclic AMP and cyclic GMP is the activation of cyclic AMP-dependent and cyclic GMP-dependent protein kinases, respectively (11). These kinases, upon binding to the appropriate cyclic nucleotide, in turn phosphorylate specific proteins, thereby regulating their performance of distinct physiological functions (14).

Receptors can either stimulate (positive coupling; e.g., prostaglandin E_1, adenosine, and β-adrenergic receptors) or inhibit (negative coupling; e.g., α_2-adrenergic and δ opiate receptors) adenylate cyclase, the enzyme which catalyzes the reaction resulting in cyclic AMP formation. The inhibition of receptor-stimulated adenylate cyclase activity by the activated δ receptor is well established, and is the subject of several recent reviews (15-17). (For a discussion of guanylate cyclase and the δ receptor, see Section II.) In brief, studies performed in intact or homogenized murine neuroblastoma and neuroblastoma x glioma clones demonstrate that opiates (18-21) and enkephalins (20-23) partially inhibit the stimulation by prostaglandin E_1 of cyclic AMP formation. In all of these studies, the opiate/opioid inhibition is antagonized by naloxone. In addition, morphine inhibits the adenosine-stimulated formation of intracellular cyclic AMP in neuroblastoma x glioma hybrids (24).

However, partial inhibition of receptor-stimulated cyclic AMP formation is not unique to the δ opiate receptor (15-17), as the same effect is induced by acetylcholine and carbamylcholine (via muscarinic cholinergic receptors) and noradrenaline (via α_2-adrenergic receptors) on prostaglandin E_1-stimulated intracellular cyclic AMP formation in 108CC15 (i.e., NG108-15) and 108CC25 neuroblastoma x glioma cells (25, 26). In clone N1E-115, carbamylcholine inhibits both prostaglandin E_1- and adenosine-stimulated intracellular cyclic AMP formation (27), as do carbamylcholine and acetylcholine in neuroblastoma cells (28). Furthermore, evidence has been obtained that the inhibitory effects of α_2-adrenergic, muscarinic acetylcholine, and opioid receptors in NG108-15 neuroblastoma x glioma cells are not additive, suggesting that the three receptors function via a single group of adenylate cyclase proteins or intermediate modulators (29).

In addition to the inhibition of adenylate cyclase function induced upon acute exposure of clones to opiates/opioids, the δ receptor mediates an enhancement of activity upon chronic exposure. This aspect of the dual regulation by opiates is demonstrated in NG108-15 neuroblastoma x glioma hybrids exposed to 10 μM morphine for 12 or more hours (30, 24). When treated cells are assayed in the absence of morphine or in the presence of an opiate antagonist, the receptor-stimulated adenylate cyclase activity and intracellular cyclic AMP level are as much as 2-fold and 4-5-fold higher than normal, respectively, although the number of δ receptors remains unchanged. If exposed cells are tested in the presence of morphine, the levels of receptor-stimulated enzyme activity and cyclic AMP appear, in effect, normal. After removal of morphine from incubation medium, the enhanced levels of activity and cyclic nucleotide gradually return to normal (30, 24). These enzymatic adaptations are considered the biochemical bases of opiate tolerance and dependence, followed by withdrawal. Confirmation that these results are not due to morphine-induced loss of δ receptor sites (down-regulation) is given by reports that treatment of neuroblastoma cells with 1 μM morphine for 24 hours does not change δ receptor binding properties (10) and that exposure of N4TG1 cells to 10 μM or 50 μM morphine for 4 hours does not decrease the δ receptor number (31).

Dual regulation of prostaglandin E_1-stimulated intracellular cyclic AMP formation and adenylate cyclase activity by opioids is seen in neuroblastoma x glioma hybrid cells 108CC15 (NG108-15) upon incubation with 1 μM met^5-enkephalin or 1 μM leu^5-enkephalin within 1-3 hours (32) and after exposure to 10 μM met^5-enkephalin or 1 μM etorphine for 12 or more hours (33). Complications arise in the interpretation of these two studies as

down-regulation is more likely to occur upon chronic exposure to high concentrations of enkephalins than of morphine, due to the higher affinity of the former agonists for the δ receptor. Thus, in N4TG1 neuroblastoma cells, incubation with 10^{-6}–10^{-8} M of an enkephalin or its derivative induces almost maximal down-regulation of δ receptors after 1 hour (31), and incubation with 0.32 μM etorphine for 20–60 minutes causes a decrease in the number of δ binding sites (34).

While the dual regulation of adenylate cyclase in cultured cells is widely accepted as the biochemical basis for opiate addiction, this phenomenon is not unique to the δ receptor (35). Carbachol (via the muscarinic acetylcholine receptor) increases the basal and prostaglandin E_1-stimulated adenylate cyclase activity in 24–30 hours in NG108-15 cells in a similar fashion to the opiate/opioid-induced states of tolerance and dependence, followed by withdrawal (36, 37). Norepinephrine (via the α_2-adrenergic receptor) induces comparable actions in 10 or more hours in the same clone (38, 39). Down-regulation of the muscarinic receptors occurs while the cells are developing the tolerant/dependent state, and recovery of the receptors upon removal of agonist from the incubation requires protein synthesis (36, 37).

In summary, the phenomenon of increased adenylate cyclase activity in compensation for constant, acute inhibition produced during chronic stimulation of a negatively coupled receptor is not a specific reaction occurring only during δ receptor-mediated opiate addiction (there are no reports of similar studies with opiates and μ receptors). Alternatively, dual regulation of adenylate cyclase by the δ, muscarinic, and α_2-adrenergic receptors in cultured cells may be a general intracellular, homeostatic response to desensitization of membranous polypeptide-hormone/neurotransmitter receptors upon chronic exposure to high concentrations of agonist (or, in some cases, down-regulation of receptors under these conditions).

II. Delta receptor coupling to guanylate cyclase

As is true of receptors in general, more is known about the interaction of the δ receptor with adenylate cyclase than of its coupling to guanylate cyclase, the enzyme which catalyzes the reaction resulting in intracellular cyclic GMP formation. Minneman and Iversen (40) first demonstrated the ability of met[5]- and leu[5]-enkephalin to stimulate intracellular cyclic GMP accumulation using a radioimmunoassay to analyze extracts from slices of rat neostriatum exposed to these opioids *in vitro* in the presence of bacitracin, a peptidase inhibitor. Due to the heterogeneity of the opiate receptors in the tissue and to the ability of the enkephalins to bind to a number of opiate receptor subtypes (2–4), however, these results cannot be attributed solely to activation of δ receptors.

In neuroblastoma clone N1E-115, met[5]-enkephalin at a final concentration sufficient to produce maximal inhibition of prostaglandin E_1-stimulated intracellular cyclic [^{3}H]AMP formation, has no effect on intracellular cyclic [^{3}H]GMP synthesis when cells are stimulated in the absence of a phosphodiesterase inhibitor (23). However, when a radioimmunoassay is employed for cyclic GMP determinations, (D-Ala[2], D-Leu[5])-enkephalin and etorphine are found to be equipotent in their ability to maximally increase the intracellular cyclic GMP content of N4TG1 cells after preincubation with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (reportedly unnecessary for synthesis of statistically significant levels of cyclic GMP) (34). Preliminary experiments also indicate that a large dose of etorphine stimulates production of a significant level of intracellular cyclic GMP in N1E-115 neuroblastoma and PC12 pheochromocytoma cells.

From available data, therefore, the δ receptor appears to be positively coupled to guanylate cyclase.

III. Induction of regulation or mobility of the delta receptor

Receptor regulation is a mechanism of cellular response to the environment, e.g., the reaction of a cell bearing neurotransmitter receptors to the concentration of a particular drug or neurotransmitter in the immediate surroundings. Regulation typically occurs with hormone and neurotransmitter receptors after prolonged or excessive exposure to agonist (41, 42), upon renewed exposure. It can take the form of acute desensitization (loss of sensitivity to an agonist) only, or, in addition, down-regulation (a decrease in number of receptors on the

cellular membrane). Desensitization can be either specific (affecting only one type of receptor on the cellular surface) or non-specific (affecting all receptors). It should be noted, however, that for many neurotransmitter receptors, desensitization may be a normal consequence of receptor activation and the mechanism of terminating the signal from the presynaptic neuron.

Receptor regulation is exemplified in N1E-115 cells, an adrenergic clone of murine neuroblastoma, by short-term desensitization of histamine H_1 and muscarinic acetylcholine receptors (43, 44) and down-regulation of muscarinic receptors (45). Acute desensitization of the histamine H_1 and muscarinic receptors is comparable in that loss of function for both receptors (which stimulate intracellular cyclic GMP formation upon binding of agonist) is agonist-mediated, specific, of relatively short duration both in inducibility and in reversibility, and temperature dependent. The mechanism for desensitization is therefore postulated to be similar (42) and to be related to the demonstrated calcium requirement for stimulation of intracellular cyclic GMP formation (46). In studies with the muscarinic receptor, the possibility was explored that calcium channels, thought to be triggered upon receptor-activation allowing calcium to enter and stimulate intracellular guanylate cyclase, become inoperative during short-term desensitization (47). Recent studies employing intracellular deposits of the photoprotein aequorin, which fluoresces upon binding of calcium ions (48), demonstrate no calcium entry subsequent to muscarinic receptor activation in intact N1E-115 cells (49), however. In addition, organic calcium channel antagonists such as nitrendipine prevent neither histamine H_1 nor muscarinic acetylcholine receptor activation of intracellular cyclic GMP formation. Studies are currently being performed to examine the necessity of Ca^{++} in cellular surface phenomena which might play a role in coupling the activated receptor to guanylate cyclase.

Analogously, evidence has been obtained in murine neuroblastoma cells that the δ receptor undergoes desensitization and down-regulation. N4TG1 cells suffer a naloxone antagonized loss in sensitivity to etorphine stimulation of intracellular cyclic GMP synthesis upon pre-incubation with that alkaloid (34). The extent of this specific desensitization depends upon the concentration of etorphine and upon the length of incubation. In addition, maximal etorphine-induced desensitization of cyclic GMP stimulation (at 0.32 μM etorphine for 7 minutes) does not modify the binding properties of [^{3}H]etorphine to intact cells, although incubation of the clone for a long duration (20–60 minutes) with the same concentration of etorphine significantly decreases the number of δ binding sites.

With the same cells, a decrease in the number of δ receptors occurs after prolonged (4 hours) incubation with met^5- or leu^5-enkephalin or an analogue (at 10^{-6}–10^{-8} M) (31). This reduction, as measured by binding of [^{125}I](D-Ala^2, D-Leu^5)-enkephalin to cellular membrane particulate fractions, is time, temperature, and dose dependent, and does not occur when non-opioids are used in the pre-incubation. The δ receptors remaining after down-regulation exhibit no change in binding affinity.

Both acute desensitization and down-regulation of δ receptors are seen in NG108-15 neuroblastoma x glioma hybrids (50). A short exposure (2 hours) of intact cells to 10 nM etorphine decreases both the ability of etorphine to inhibit prostaglandin E_1-stimulated adenylate cyclase activity and the binding affinity of [^{3}H]diprenorphine to whole cells, although no reduction in binding occurs. A longer incubation (24 hours) with the identical concentration of etorphine causes both a total inability of etorphine to inhibit prostaglandin E_1-stimulated adenylate cyclase activity and a reduction in the number of [^{3}H]diprenorphine binding sites, with no change in binding affinity.

The δ receptor, therefore, upon interaction with agonist under appropriate conditions, is regulated in the same manner demonstrated for a number of other hormone and neurotransmitter receptors. In terms of mobility on the cellular membrane, however, the activated δ receptor demonstrates a pattern which is unique.

A recent review (51) classifies soluble polypeptide-binding receptors on mammalian plasma membranes (as opposed to receptors for steroid hormones and neurotransmitters) into two general categories based on function: one class consists of receptors whose biological effects are initiated immediately upon binding of polypeptide agonist, and the second group contains receptors which accomplish their function (mediation of transcellular movement of macromolecules) by first becoming internalized with bound agonist. In the first class of

polypeptide receptors, to which the δ receptor belongs, are those with hormone agonists such as epidermal growth factor, insulin, glucagon, etc., which are typically controlled by down-regulation. Upon agonist binding, these receptors aggregate into clusters on the surface of the cellular membrane, the clusters ultimately being internalized presumably via coated pits. After internalization, the receptors are eventually degraded intracellularly. (Receptors in the second group, after internalization and separation from agonist, appear to be recycled to the cellular surface and re-utilized.)

While clustering and internalization might appear to be the first steps of down-regulation, there is not support for that conclusion by the actions of polypeptide-hormone receptors currently known to undergo down-regulation (see review 52). (There is, however, some suggestion that clustering and/or internalization of polypeptide-hormone receptor complexes are prerequisite for initiating some long-term intracellular effects (for more detailed reviews, see 52, 53, and 54).) The δ opioid receptor is, in fact, a case against a connection between internalization and down-regulation.

As demonstrated with a rhodamine-labeled derivative of leu^5-enkephalin and image intensified fluorescence microscopy, the δ receptor, upon binding of agonist, forms clusters on the surface of N4TG1 neuroblastoma (55) and NG108-15 neuroblastoma x glioma hybrid cells (56). However, unlike all other polypeptide-hormone receptors studied (52), no internalization of the δ receptor clusters occurs in either clone. In addition, clusters on the cellular membrane virtually disappear after exposure to 100 mM sodium or washing under conditions which dissociate bound agonist from δ receptors (55, 56). The use of sulfhydryl and disulfide reagents during pretreatment of the N4TG1 clone demonstrates that formation of clusters is not necessary either for binding of [^{125}I] (D-Ala^2, D-Leu^5)-enkephalin to whole cells (55) or for agonist inhibition of receptor-stimulated adenylate cyclase activity (57).

Thus, clustering of activated δ receptors on the cellular surface is not followed by receptor internalization. However, the significance of the clustering phenomenon is unknown at this time, as aggregation is apparently not required for binding or function of the δ receptor. Agonist-induced down-regulation of δ receptors also appears to follow a pathway independent of clustering.

Finally, the maximum number of δ receptor sites per milligram of membrane protein (B_{max}) is regulated in neuroblastoma cells, during the process of cellular division. In clone N1E-115, the B_{max} increases two-fold as cells progress from logarithmic to stationary phase (23). The δ receptors exhibit no change in the value of the apparent equilibrium dissociation constant (K_D) for [^{3}H]met^5-enkephalin, however, during the course of the growth cycle.

IV. Membranous lipids and delta receptor function

As no agonist or antagonist specific for the δ receptor has yet been discovered or synthesized, interaction of the δ receptor with adjacent membranous lipids is more selectively studied in neuroblastoma cells than with membranes or whole cells originating in the central or peripheral nervous system. Should the cellular membrane in a neuroblastoma clone bind enkephalin, any resulting changes can be attributed solely to activation of the δ receptor. This is not true for nervous tissue due to the heterogeneity of the opiate receptors and the ability of enkephalins or enkephalin analogues to bind to a number of opiate receptor subtypes (2–4). The ultimate results therein will be influenced by the ratio of the multiple types of opiate/opioid receptors and the relative affinity of the ligand for each subtype, unless assay conditions are modified in a non-physiological fashion, e.g., inclusion of sodium cholate or alcohol to differentiate μ and δ receptors (58, 59).

Relatively little is known about the interaction of activated δ receptors with constituents of the surrounding cellular membrane or of the mechanism by which activated δ receptors couple with the effectors guanylate cyclase and adenylate cyclase. Studies assessing the effect on δ receptors of controlled membranous modifications have been done with fatty acids and NG108-15 neuroblastoma x glioma hybrids. Cells cultured in lipid-free medium containing different, additional, saturated fatty acids of varying lengths demonstrate no change in the maximum number of binding sites or in the affinity for radiolabeled agonists. However, cells grown in lipid-free medium containing various fatty acids of identical lengths but increasing unsaturation demonstrate decreasing values for B_{max}, no change in affinity, and a corresponding change in

the fatty acid composition of membranous phospholipids (60). Further investigation of the clone under equivalent growth conditions indicates that in spite of the above modifications resulting from exposure to unsaturated fatty acids, the ability of etorphine to inhibit prostaglandin E_1-induced adenylate cyclase activity is not changed (61).

Incubation of NG108-15 cells for 2 days in lipid-free medium supplemented with the unsaturated fatty acid linoleic acid results in a differentiation between the compensatory increase in adenylate cyclase activity induced by chronic stimulation of the δ receptor and of the α_2-adrenergic receptor. While growth of cells in serum lipids allows the subsequent expression of both δ and α_2-adrenergic receptor-induced states of tolerance, morphine-induced tolerance requires a minimal addition of linoleic acid to lipid-free growth medium, whereas norepinephrine-induced tolerance occurs when cells are cultured in the complete absence of lipids (62). In contrast, acute inhibition of adenylate cyclase activity can be induced by either morphine or norepinephrine in cells grown in lipid-free medium.

While differing data exist as to the effect it has on δ receptor function, an increase in the proportion of unsaturated fatty acids in a cellular membrane, all else remaining constant, generally results in an increase in the fluidity of the membrane. Such a change would be predicted to affect the functional coupling of any receptor to its membranous effector.

In contrast to the above studies, incubation of intact NG108-15 cells with phospholipase C, an enzyme which removes the phosphorylated head group from phospholipids in the membrane, results in a reduction in the binding affinity of the δ receptors for a radiolabeled enkephalin analogue with no change in the number of binding sites, and in a decrease in the ability of leu^5-enkephalin to inhibit prostaglandin E_1-induced adenylate cyclase activity (63). These results suggest that integrity of the phospholipids in the cellular membrane is important for the function of the δ receptor.

Other studies have been performed on the relationship of δ receptor function to membranous glycolipids. The δ receptors demonstrated in N18TG2 cells (the parent neuroblastoma clone of NG108-15 neuroblastoma x glioma hybrids) produce only a slight inhibition, upon morphine or met^5-enkephalin stimulation, of prostaglandin E_1-induced adenylate cyclase activity (64, 65). However, incorporation of cerebroside sulfate into the cellular membrane by including the compound in incubation medium for a short time results in a potentiation of the ability of morphine and met^5-enkephalin to inhibit prostaglandin E_1-stimulated intracellular cyclic AMP production (65, 66). No significant change is seen in the δ receptor number or in the binding affinity for a radiolabeled enkephalin analogue. However, the correlation between δ receptor activity and the content of cerebroside sulfate in cellular membranes is not simple, as lack of cerebroside sulfate does not prevent, and addition of cerebroside sulfate does not enhance, the binding (and, by inference, the function) of a radiolabeled enkephalin to the δ receptors in N4TG1 neuroblastoma cells (67).

The most well-defined interaction of the activated δ receptor with membranous lipids is the naloxone antagonized, dose-dependent reduction in synthesis of total sialoglycosphingolipids (gangliosides) and membrane glycoproteins observed upon 24 hours incubation of either N4TG1 neuroblastoma cells (68, 69) or NG108-15 neuroblastoma x glioma hybrids (69) with 10^{-6}–10^{-10} M opiates (morphine or levorphanol) or opioids (β-endorphin or an enkephalin analogue). No change in cellular division or synthesis of other protein and glycoprotein, DNA, membranous phospholipid, or proteoglycan is observed. Further addition of opiate/opioid after the initial 24 hours incubation results in a continuation of the inhibition, which, otherwise, gradually ceases (69). In addition, exposure of morphine- or enkephalin analogue-treated cells to compounds which induce intracellular cyclic AMP formation stimulates the synthesis of gangliosides and membrane glycoproteins, resulting in a reversal of the opiate/opioid-induced inhibition (69). The suggestion was made that opiate/opioid-induced acute inhibition of receptor-stimulated intracellular cyclic AMP formation in cultured cells is functionally related to the decrease in ganglioside and membrane glycoprotein synthesis, presumably via inhibition of cyclic AMP-induced phosphorylation of glycosyltransferases (69).

However, rationalizing this hypothesis with other data obtained in neuroblastoma or neuroblastoma x glioma clones is not straightforward. In the described experiments, no opiate/opioid-induced inhibition could be detected in N1E-115 neuro-

blastoma cells, a clone demonstrated to have functional δ receptors (10, 23). The conditions of incubation employed with the opiates/opioids probably induce tolerance in NG108-15 cells, a state in which adenylate cyclase activity is raised to a level compensatory for the acute inhibition induced by agonists (see Section I). Furthermore, similar incubation with enkephalin analogue induces down-regulation of δ receptors in N4TG1 cells (31), introducing the complication of relating the enkephalin analogue-induced inhibition of ganglioside/membrane glycoprotein synthesis to a loss of δ receptors by down-regulation; there are, however, no reports of morphine-induced down-regulation of δ receptors in cultured neuroblastoma or neuroblastoma x glioma cells.

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