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Reversible Modulation of Opioid Receptor Binding in Intact Neural Cells by Endogenous Guanosine Triphosphate

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SUMMARY

Incubation of SH-SY5Y neural cells with mycophenolic acid (MPA), an inhibitor of inosine monophosphate dehydrogenase (the key enzyme in purine nucleotide biosynthesis), reduced the cellular content of GTP by 94% relative to its concentration in control cells (43 nmol/mg protein) without altering the level of GDP. Although in GTP-depleted intact cells the receptor binding parameters (K_d and B_{max}) of the opioid antagonist [^{3}H]naltrexone were unchanged from those in untreated cells, the binding affinity of the μ-selective opioid agonist [^{3}H]Tyr-D-Ala-Gly-(Me)-Phe-Gly-ol ([^{3}H]DAMGO) was enhanced 2-fold. Furthermore, the kinetics of ligand/receptor interaction revealed that in the nucleotide-depleted cells, the dissociation rate constant for [^{3}H]DAMGO was reduced by 44%. Initial exposure of SH-SY5Y cells to pertussis toxin reduced high-affinity ligand binding by 95% and abolished the effect of MPA treatment. Renewed incubation of the GTP-depleted cells with guanosine restored the original GTP levels and agonist binding. Neither MPA nor guanosine treatment changed the B_{max} of [^{3}H]DAMGO binding. Forskolin- and prostaglandin E_1-stimulated adenylyl cyclase activities were decreased significantly in GTP-depleted cells. DAMGO and [D-Pen^2,D-Pen^5]enkephalin inhibitions of adenylyl cyclase were also affected with MPA treatment. Maximal inhibition of forskolin-stimulated adenylyl cyclase activity by both of the agonists was reduced, whereas MPA caused a 2-fold reduction in potency for DAMGO. The results show that reduction in endogenous GTP levels leads to noticeable changes in agonist, receptor, and G protein interactions, as measured by agonist binding, and to subsequent diminution of the signal transduction, as reflected by the cAMP levels.

Modulation by guanine nucleotides was one of the early recognized regulatory mechanisms of opioid receptor binding (1). In numerous studies with isolated neural membranes, the ability of GTP, or its nonhydrolyzable analogues, to selectively reduce the binding affinity of opioid agonists was described (e.g., Refs. 2 and 3). In brain membranes, the reconstituted ternary complex of ligand/receptor/G protein exhibited high affinity opioid agonist binding, whereas elimination of G protein, or GTP-induced dissociation of the complex, strongly reduced receptor binding affinity (4). Concurrently, it was shown that the multiphasic binding of opioid antagonist is due to its interactions with different opioid receptor types (5). In contrast to this level of understanding obtained with isolated neural membranes, the modulation of opioid receptor in whole cells by endogenous guanine nucleotides has not been demonstrated. To address this, we attempted to reversibly alter the GTP content of viable, nonleaking SH-SY5Y neural cells and to determine the consequences of such treatment on the receptor binding of μ-selective opioid agonists and antagonists in the intact cells. The effectiveness of the antitumor agent MPA in inhibiting the biosynthesis of GTP without appreciably altering the cell content of GDP and of adenine nucleotides has previously been described (6, 7). The results of the present study reveal a regulatory role for endogenous GTP in modulating opioid receptor/G protein coupling in neural cells and, thus, the binding affinity (4) and signal transduction (8) of opioids.

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Experimental Procedures

Materials. [^{3}H]DAMGO (60 Ci/mmol) was obtained from Amersham, and [^{3}H]naltrexone (22 Ci/mmol) was kindly provided by the National Institute on Drug Abuse. The biochemicals, including Dulbecco's modified Eagle's medium, were purchased from Sigma Chemical Co., and fetal calf serum was obtained from GIBCO.

Cell culture and treatment. SH-SY5Y neural cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. For the controls, the cells were washed three times with isotonic phosphate-buffered saline (150 mM NaCl, 0.51 mM Na_2HPO_4, 0.38 mM KH_2PO_4, pH 7.4); lifted from the flask surface by incubation with a Ca^{2+}, Mg^{2+}-free Puck's solution for 10 min (9, 10); and pelleted by centrifugation at 200 × g for 5 min. For cell treatment, either 30 μM MPA alone or combined with 500 μM guanosine was added to the growth medium. After 5 hr, the cells were washed

ABBREVIATIONS: DAMGO, Tyr-D-Ala-Gly-(Me)-Phe-Gly-ol; MPA, mycophenolic acid; PTX, pertussis toxin; DPDPE, [D-Pen^2,DPen^5]enkephalin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

three times with phosphate-buffered saline, collected by centrifugation, and suspended in appropriate buffers for determination of GTP, Na^+, and K^+ or for ligand binding. An alternative, equally effective, treatment of the cells consisted of initial incubation with MPA for 3 hr, followed by renewed incubation with guanosine for 3 hr.

Cells were differentiated with 10 μM retinoic acid for 6 days, with the medium changed every 3 days; these cells were used for adenylyl cyclase estimations.

Protein determination. Aliquots of the cell suspension were treated with 1 N NaOH at room temperature, and protein content was estimated with bovine serum albumin as standard (11). The ratio of cell count to cellular protein was 3.5×10^6 cells/mg cell protein.

Measurement of intracellular guanine nucleotides. The washed cells were sonicated in 0.6 N trichloroacetic acid on ice and then centrifuged at 10,000 × *g* for 10 min. The supernatant was extracted with 3 volumes of diethyl ether to remove excess trichloroacetic acid, and the concentrations of GTP and GDP were determined by high performance liquid chromatography with an anion exchange column (250 mm × 5 mm, Supelcosil LC-SAX) with a gradient of buffers A (10 mM acetic acid, 6 mM KH_2PO_4, pH 4.0) and B (600 mM KH_2PO_4, pH 7.0) at a flow rate of 1 ml/min. Absorbance was measured at 260 nm (12).

Determination of cellular K^+ and Na^+. Cells were pelleted by centrifugation and washed four times with ice-cold physiological buffer (9) in which sodium was replaced with equimolar choline (118 mM choline chloride, 1.3 mM $CaCl_2$, $2H_2O$, 3 mM $MgSO_4$, $7H_2O$, 10 mM glucose, 20 mM HEPES buffer, pH 7.4). The washed pellet was suspended in 15 mEq/l $LiNO_3$ (internal standard), 70% HNO_3 was added, and the capped polypropylene tubes were heated at 70° for 2 hr. Subsequently, the samples were subjected to flame photometry as previously described (13).

Ligand binding. Cells were washed with ice-cold phosphate-buffered saline and lifted off the surface by incubation with modified Puck's solution (14). The cell suspension was then centrifuged for 4 min at 200 × *g*, and the pellet was resuspended in the physiological buffer medium (see above) at a concentration of 0.5 mg protein/ml. Aliquots of this suspension (200–400 μl) were included in the assay medium that, in a total volume of 500 μl, also contained various concentrations of radiolabeled opioid. Nonspecific binding was determined with 5 or 1 μM unlabeled DAMGO or naltrexone, respectively. After incubation for 50 min at 37° to achieve binding equilibrium of [^{3}H]DAMGO or [^{3}H]naltrexone, the samples were quickly filtered and subjected to liquid scintillation counting (9). To determine the rates of ligand association with the receptor, we incubated the cells for various lengths of time with [^{3}H]DAMGO, followed by quick filtration. To assess ligand dissociation from receptor, we incubated the cells with [^{3}H]DAMGO to reach equilibrium in binding; then, an excess of unlabeled DAMGO was added to initiate the dissociation. At frequent time intervals afterward, the assay medium was filtered, and the radioactivity on the filters was measured. The experiments on ligand association and dissociation were carried out with a concentration of [^{3}H]DAMGO that, as indicated by the corresponding K_d value (Table 1), predominantly bound to the high affinity component of μ receptor.

Adenylyl cyclase assay. SH-SY5Y cells differentiated with retinoic acid were collected and resuspended in the physiological medium with 8 mM theophylline. After 5 min at 37°, acute inhibition of adenylyl cyclase activity was done essentially as described earlier (10) in a final volume of 100 μl in the presence of 10 μM forskolin/PGE1 and various concentrations of DAMGO. Incubation was stopped with 50 μl of 0.15 N HCl, and the samples were maintained at 70° for 60 sec and frozen at −80°. cAMP estimations were carried out, after adjustment of pH, with a radioligand binding assay kit from Diagnostic Product Corporation.

Data analysis. Binding data were fitted to a one- and two-site model with or without a linear binding component by nonlinear regression analysis using the NONLIN module of the computer program SYSTAT (14). The best fit to a given model was determined using the F-ratio test to compare the weighted residual sum of squares with $p > 0.05$. The corresponding standard error was computed within NONLIN from the residual sum of squares of the regression (9). The data on ligand association and dissociation were fit to a nonlinear regression program (Graph Pad Software, San Diego, CA) to yield the values for k_{+1} and k_{-1} (15). Nonlinear regression analysis of data for inhibition of adenylate cyclase was also computed with Graph Pad.

Results

Guanine nucleotide content. By reducing the *de novo* synthesis of GTP with MPA, a potent inhibitor of the biosynthetic enzyme inosinate dehydrogenase, the content of this nucleotide in SHSY5Y cells was decreased by 85–90% relative to its concentration in control cells (Fig. 1). The determined concentration of GTP in control SHSY5Y cells (43 nmol/mg protein) was similar to those measured in cells from rat kidney (6), cultured murine lymphoma, and mouse neuroblastoma (7). Concurrently, maximal changes in the cellular contents of the triphosphates of adenosine, uridine or cytosine, were 10–15%. The possible leakiness of the plasma membrane was monitored and excluded throughout the experiments implemented in the present study. Thus, the measured GTP concentration in control and MPA- and guanosine-treated cells represents the true steady state at which receptor binding in intact cells was determined. Guanosine, when included in the medium, returned the GTP content to normal levels.

Agonist and antagonist binding. Saturation binding of agonist [^{3}H]DAMGO yielded a K_d of 4.0 ± 0.60 nM and B_{max} of 43.6 ± 6.02 fmol/mg in control cells. MPA-treated cells exhibited a 2.7-fold increase in affinity (K_d of 1.5 ± 0.23 nM), whereas B_{max} remained the same (Fig. 3) (Table 1). (^{3}H)-

TABLE 1

Parameters of [^{3}H]DAMGO and [^{3}H]naltrexone binding in intact cells

The experimental conditions under which these parameters were determined are described in the legends to Figs. 2–4. The listed values were obtained by nonlinear regression analysis, as described in Methods. Shown are mean ± standard error values obtained in number of experiments (in parentheses).

Conditions	K_d	B_{max}	k_{assoc}	k_{dissoc}
	nM	fmol/mg	$min^{-1}\ M^{-1}$	min^{-1}
[^{3}H]DAMGO binding				
Control cells	4.00 ± 0.60 (4)	43.63 ± 6.02 (4)	3.33×10^7 ± 0.03 (3)	15.5×10^{-2} ± 0.02 (3)
MPA treatment	1.49 ± 0.23 (4)	43.97 ± 3.76 (4)	2.96×10^7 ± 0.06 (3)	8.7×10^{-2} ± 0.06 (3)
MPA and guanosine treatment	3.95 ± 0.82 (4)	44.04 ± 4.20 (4)		
[^{3}H]Naltrexone binding				
Control cells	0.36 ± 0.05 (4)	159.5 ± 8.6 (4)		
MPA treatment	0.49 ± 0.09 (4)	146.0 ± 5.3 (4)		

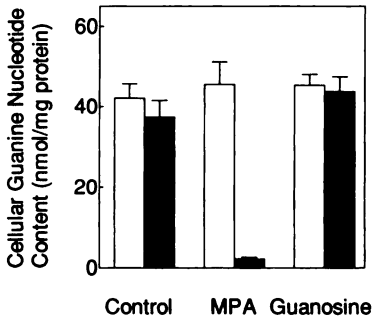

Fig. 1. Concentrations of GTP and GDP in control and MPA-modified SH-SY5Y cells. The cells were cultured in the absence of agents or in the presence of MPA (30 μM), guanosine (500 μM), or both for 5 hr. The collected cells (from four or five flasks) were washed with cold phosphate-buffered saline and processed for guanine nucleotide quantification by high performance liquid chromatography as described in Experimental Procedures. Shown are concentrations of GDP (*open bars*) and GTP (*shaded bars*), plotted as mean ± standard error values of four experiments.

Naltrexone showed K_d values of 0.36 and 0.49 and B_{max} values of 159 and 146 fmol/mg in control and treated cells, respectively (Fig. 2). Equilibrium dissociation constants obtained for [^{3}H]DAMGO in the present study are comparable to those of the high affinity receptor in SH-SY5Y cells as reported previously (10). Because G protein confers high affinity on the receptor (4), it was surmised that any changes in cellular GTP levels would affect only this population of receptor, and therefore no attempts were made to study the low affinity sites. It should be noted that saturation binding experiments do not detect the receptor of low affinity (K_d >0.1 μM).

Because the binding assays were performed in physiological buffer containing 118 mM sodium, the B_{max} of agonist was lower than that for the antagonist. Sodium inhibits agonist binding to several receptors that are coupled to G_i/G_o (16). Furthermore, agonist binding to intact cells is attenuated by the endogenous guanine nucleotides (17).

Incubation of cells with guanosine in combination with MPA increases cellular GTP levels, and as a result, the affinity of the receptor reverted to its original state as in

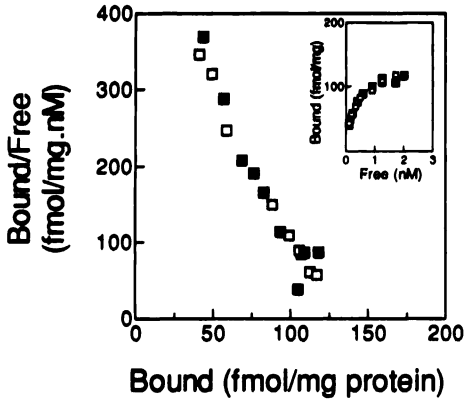

Fig. 2. Opioid antagonist binding in SH-SY5Y cells before and after GTP depletion. Equilibrium binding of [^{3}H]naltrexone in intact cells was determined in physiological buffer at 37° as described in Experimental Procedures and in the legend to Fig. 3. Shown are saturation binding (*inset*) and the corresponding Scatchard plots in control (□) and MPA-treated (■) cells. Plotted are the results of representative experiments replicated four times. The corresponding binding parameters and data variability are listed in Table 1.

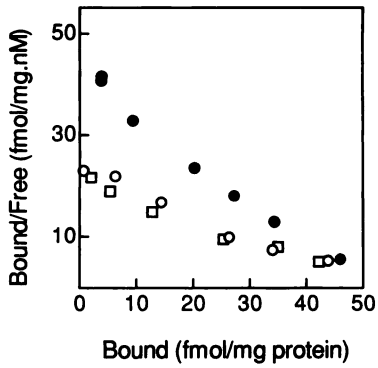

Fig. 3. Opioid agonist binding in control, MPA-treated, and guanosine-treated SH-SY5Y cells. Equilibrium binding of [^{3}H]DAMGO was determined in control cells (○) and cells initially exposed to 30 μM MPA (●) or both 30 μM MPA and 500 μM guanosine (□) for 5 hr. After treatment, the surface-attached cells were thoroughly washed by aspiration with isotonic phosphate-buffered saline, lifted from the flask surface, suspended in physiological buffer, and included in the radioligand binding assay. Shown are results of representative experiments replicated four times. The corresponding binding parameters and statistical assessment are listed in Table 1.

control cells (Fig. 3). These effects caused by MPA were specific due to GTP depletion of the cells because MPA addition *per se* did not influence the agonist binding in membranes. Similar effects could not be demonstrated for DPDPE binding to δ-opioid receptors as these sites are expressed low in SH-SY5Y cells. Pertussis toxin abolished the enhancement of high affinity binding in GTP-depleted cells and rendered ligand binding in the control and MPA-treated cells virtually identical (Fig. 4).

To understand the mechanistic aspects involved in the enhancement of the high affinity component of saturation isotherms, association and dissociation rate constants were computed. Although the binding of [^{3}H]DAMGO in MPA-treated cells was twice that in control cells, association rates were similar (Table 1). These data could be fit to a single set of binding sites, indicating the homogeneous population of receptors at the concentration of [^{3}H]DAMGO used (1–3 nM)

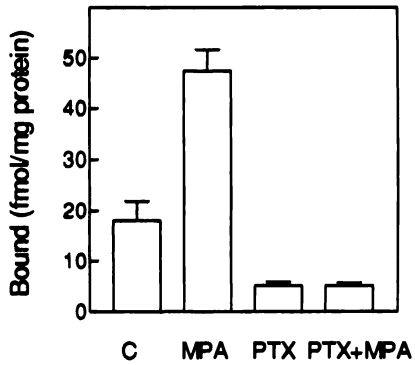

Fig. 4. Opioid agonist binding in control and MPA-treated cells after their initial exposure to PTX. SH-SY5Y cells in culture were exposed to PTX (80 ng/ml) for 24 hr as described (10). Subsequently, the cells were incubated in the absence and presence of MPA (30 μM) for 5 hr. Other flasks of cells were exposed to MPA only or incubated without any added agent. Subsequently, the cells were washed with phosphate-buffered saline and suspended in the physiological buffer described in Experimental Procedures, and the specific binding of 2 nM [^{3}H]DAMGO in the control cells (*C*) and cells treated with MPA (*MPA*), pertussis toxin (*PTX*), or both (*PTX+MPA*) was determined. Shown are mean ± standard error values of four experiments.

(Fig. 5, *top*). Dissociation followed first-order rate kinetics, and the rate in MPA-treated cells (8.7×10^{-2} min^{-1}) was significantly lower than that observed in control cells (15.5×10^{-2} min^{-1}) (Fig. 5, *bottom*) (Table 1).

Adenylyl cyclase assays. MPA treatment significantly decreased the prostaglandin E_1-stimulated adenylyl cyclase activity (from 35.65 ± 0.72 pmol/min/mg for control to 23.35 ± 2.13 pmol/min/mg; $p < 0.001$) and the forskolin-stimulated adenylyl cyclase activity (30.4 ± 2.42 pmol/min/mg to 25.1 ± 1.64 pmol/min/mg). This is in accordance with earlier reports that showed a reduction in β-adrenergic response of cultured glioma cells on depletion of intracellular GTP (18). DAMGO inhibited adenylyl cyclase activity by 75–85% in control cells, and this was reduced to 55–60% in MPA-treated cells (Fig. 6, *top*). A concomitant 2-fold increase in EC_{50} was observed (10.6 ± 1.37 to 22.2 ± 1.31 nM). Dose-response curves for DPDPE, a δ agonist, showed a reduction in maximal inhibition from 61 ± 1.6% to 44 ± 2% on GTP depletion. The potency of DPDPE was not significantly altered, although a similar trend was observed for the μ receptor. The EC_{50} values obtained in control and treated cells were 3.92 ± 1.41 and 6.88 ± 1.23 nM, respectively (Fig. 6, *bottom*).

Discussion

Guanine nucleotides are known to reduce the affinity of agonist binding to receptors coupled to heterotrimeric G proteins in membrane binding experiments (19). However, the same effect has not been examined in intact cells. In the present report we used MPA to achieve an understanding of the guanine nucleotide regulation of ligand binding and the subsequent signal transduction in intact cells by reducing cellular GTP levels. MPA has previously been used in a number of studies to elucidate the role of GTP in various biochemical functions (20–22).

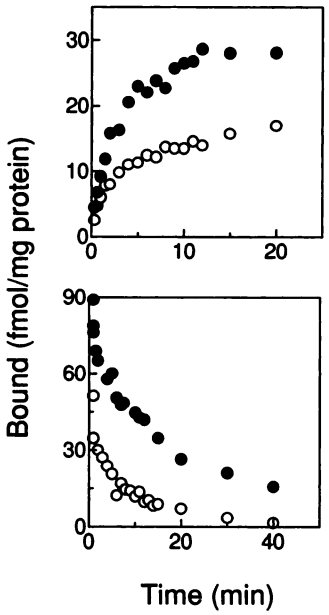

Fig. 5. Association and dissociation kinetics of opioid agonist binding in control and MPA-treated SH-SY5Y cells. *Top*, in association experiments, control cells (○) or cells initially treated with MPA (●) were suspended in the physiological buffer described in Experimental Procedures. After incubation at 25° for 10 min, 0.8 nM [^{3}H]DAMGO was added, and at various times thereafter the incubation was terminated by rapid filtration. Bottom, in dissociation experiments, control (○) or MPA-treated (●) cells, suspended in the physiological buffer, were incubated with 2 nM [^{3}H]DAMGO for 40 min. After the addition of 1 μM unlabeled DAMGO to initiate ligand dissociation from receptor, the assay medium was filtered at the times shown, and the radioactivity on the filters was determined as described in Experimental Procedures. Depicted are results of representative experiments replicated three times. The corresponding rate constants are listed in Table 1.

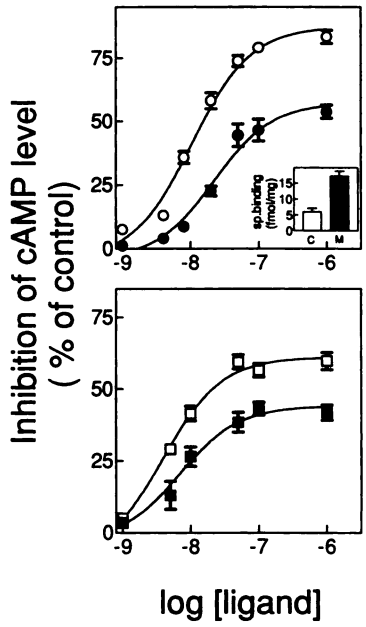

Fig. 6. Opioid inhibition of forskolin-stimulated adenylyl cyclase activity in retinoic acid-differentiated SH-SY5Y cells. Control cells (*open symbols*) or MPA-treated cells (*filled symbols*) were suspended in buffer containing 8 mM theophylline, and adenylyl cyclase activity was measured in the presence of forskolin and various concentrations of DAMGO, a μ-agonist (*top*), or DPDPE, a δ-agonist (*bottom*). cAMP estimations were carried out as described in Experimental Procedures. *Inset*, specific binding of [^{3}H]DAMGO to intact differentiated cells, at 2 nM [^{3}H]DAMGO, in control (*C*) and MPA-treated (*M*) cells.

MPA treatment selectively increased the affinity of [^{3}H]DAMGO by 2.7-fold in saturation binding experiments without altering the antagonist binding. This effect was due to the reduced cellular GTP levels because guanosine addition to the medium reversed the affinity changes as a result of recovered GTP levels. Furthermore, PTX treatment of cells abolished the MPA effect, substantiating the G protein-mediated nature of the affinity transitions that were studied.

According to the current models, ligand binding to receptor leads to dissociation of GDP from the G protein, followed by formation of ligand/receptor/G protein complex, i.e.,[LRG_e] (19). G protein is nucleotide free or empty in this state, and this ternary complex exhibits high affinity for the ligand. Under conditions of GTP abundance, [LRG_e] is removed rapidly, and the turnover rate is so fast that no appreciable [LRG_e] accumulates. Thus, in control cells, the higher affinity ternary complex has only a transient appearance. However, when GTP is limiting (as in MPA-treated cells), slower GTP binding would raise the steady state concentrations of [LRG_e], and this in turn would increase the overall affinity of the system. This view gains support from the kinetic data (Table 1). Although association rates were similar, agonist dissociation rates in MPA-treated cells were slower by 2-fold. The factor of 2 reduction in dissociation rate constant completely explains the 2-fold enhancement of the affinity of

DAMGO in MPA-treated cells. Accumulation of [G_e] has been previously shown to result from a mutation in $G_{s\alpha}$ and implicated in the gain and loss of function observed in patients with endocrine disorders (23).

GDP has been shown to be as potent as GTP in reducing high affinity agonist binding in membrane assays (2). However, GDP levels were not altered in MPA-treated cells, and the net effect noticed can be attributed only to changed GTP levels. Untreated cells exhibit binding that is attenuated by both GDP and GTP, whereas the binding in MPA-treated cells represents the component contributed by GDP to the total inhibitory effect on agonist binding. In membranes devoid of guanine nucleotides, receptor is in equilibrium with the prevalent, precoupled receptor, as indicated by the high basal activity observed in many systems (24), and the ligand binding is indicative of high affinity [LRG_e] formation. GDP addition prevents the precoupling, increases the free receptor, and facilitates the interaction of ligand/receptor complexes with the G protein (25). Therefore, the dramatic affinity changes measured in membranes on guanine nucleotide addition are due to the transitions between two states, i.e., the high affinity ternary complex [LRG_e] and GDP/GTP bound forms of [LRG] (saturation binding of [^{3}H]DAMGO to membranes in the presence of sodium gives K_d of 1.2 nM, and the addition of 1 μM GTPγS increases the K_d values to 17–20 nM[1]). In intact cells, however, various affinity states formed subsequent to ligand binding are in dynamic equilibrium, and the apparent affinity measured at any instant indicates the average affinity of all existing states. At equilibrium, a steady state is established among [LRG_{GDP}], [LRG_e], and [LRG_{GTP}] of all other possible states. Although [LRG_e] is negligible and only the guanine nucleotide-bound states represent the binding in control cells, [LRG_{GTP}] is minimal in MPA-treated cells, and an increase in [LRG_e] would increase the affinity of the system. In essence, the presence of GDP in intact cells masks the maximal effect of reduced GTP levels.

GTP exchange with GDP on G protein normally leads to dissociation of the trimer into its components: $\beta\gamma$ and α bound to GTP. Both α-GTP and $\beta\gamma$ have been shown to regulate the adenylyl cyclase activity (19). A reduction in cellular GTP levels would decrease α-GTP/$\beta\gamma$ levels and subsequently decrease regulation of the downstream effector function. Thus, a generalized effect on all G protein-coupled receptor-mediated functions can be expected. In accordance, prostaglandin E_1-stimulated cAMP levels were lowered significantly in MPA-treated cells, indicating the impairment of signal transduction through G_s protein. Forskolin-stimulated adenylyl cyclase activity was also decreased, highlighting the importance of the G_s-mediated component in forskolin activation, as reported earlier (26). There was a significant decrease in maximal inhibition of adenylyl cyclase activity by agonists to μ-opioid (DAMGO) and δ-opioid (DPDPE) receptors that are coupled to $G_{i/o}$ proteins. In addition, a 2-fold reduction in potency of DAMGO was measured. This is in contrast to earlier studies that examined the α_2 agonist inhibition of adenylyl cyclase activity in membranes at various concentrations of GTP (27). An increase in the affinity of the system is expected to enhance the potency of a drug. However, in a system with "spare receptors," functional antagonism at any step of signal transduction pathway can result in a dextral displacement of the dose-response curves. SH-SY5Y cells have been shown to have receptor reserve for DAMGO (10), and this could be the reason for the rightward shift observed in dose-response curves of DAMGO inhibition of forskolin-stimulated adenylyl cyclase activity.

Direct application of antitumor drugs such as MPA, whose effectiveness is based on the inhibition of nucleic acid synthesis, can lead to depletion of cellular GTP (28). Decreased GTP concentrations in erythrocytes of patients with deficiencies of the purine salvage enzymes, purine nucleotide phosphorylase, and hypoxanthine-guanosine phosphoryl transferase were reported (29, 30) and correlated with central nervous system dysfunction such as the Lesch-Nyhan syndrome (30). The cumulative effect of reduced GTP levels on various G protein-coupled receptors may play an important role in the pathophysiology of CNS diseases.

Considering the intracellular GTP concentrations of 0.3–0.5 mM (7, 18), it is interesting that significant changes at the receptor and effector occurred as a result of MPA treatment. In membranes, 1–10 μM GTP regulates agonist binding. However, GTP may not be uniformly distributed in the cell, and there may be a functional compartmentation of GTP (31). The presence in the cytoplasm of a separate pool of GTP for the activation of adenylyl cyclase has been proposed (20). In addition, the effective local concentration of GTP is dependent on the abundance of NDPkinase that catalyzes the transphosphorylation reactions (32).

The results of the present study highlight the importance of various affinity states that are formed subsequent to agonist binding (3) and provide evidence for the formation of a high affinity state of the receptor in intact cells during signal transduction. Noticeable changes in the affinity of agonist binding and alterations in DAMGO- and DPDPE-mediated inhibition of cAMP levels indicate that separate pools of cellular GTP probably exist and that ambient GTP content in these receptor/G protein domains is vulnerable to the action of antitumor drugs such as MPA.

Acknowledgments

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