MODULATION OF OPIOID SIGNAL TRANSDUCTION IN SH-SY5Y NEURAL CELLS BY DIFFERENTIATING AGENTS: CONCURRENT MU RECEPTOR UP-REGULATION AND EFFECTOR DESENSITIZATION BY PHORBOL ESTER

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ABSTRACT: Long-term exposure of SH-SY5Y human neural cells to retinoic acid (RA) increased the binding of [^{3}H]DAMGO and [^{3}H]DPDPE by up to 3-fold compared to membranes of untreated cells. In contrast, incubation of the cells with phorbol ester (TPA) selectively up-regulated the binding of [^{3}H]DAMGO. RA enhanced the maximal inhibition of cAMP formation in SH-SY5Y cells by both DAMGO and DPDPE from 20% for both opioids in control cells to 75% and 50%, respectively. On the other hand, the effect of TPA was limited to a marginal increase in the inhibition of cAMP formation by DAMGO. Parameters of GTPγ^{35}S binding as well as low-Km GTPase activity revealed no deterioration in the structure or function of total G protein in the TPA-treated cells, and initial Western blots showed no difference in the cell content of G_o. Ongoing experiments are focusing on the covalent modification of specific G protein subtypes involved in μ and δ opioid signal transduction in SH-SY5Y cells differentiated by TPA.

METHODS: Human neuroblastoma cells SH-SY5Y were cultured and differentiated with RA or TPA for 9-11 days as described (1). Ligand binding was resolved and quantified by computer fitting using the statistical program SYSTAT, and the activities of adenylyl cyclase and GTPase were determined by a radioligand binding assay and measurement of released ^{32}P, respectively (2). Binding of GTPγ^{35}S (3), Western blotting (4), and the determination of protein (3) were implemented as described previously.

RESULTS AND DISCUSSION: The SH-SY5Y human neural cells were shown to be susceptible to functional differentiation by various inducers, including RA and TPA (5). It was also demonstrated that exposure of these cells to RA enhanced the inhibition of adenylyl cyclase (AC) by μ opioid agonists (6,7). The molecular characteristics of opioid signal transduction through receptors, G protein and adenylyl cyclase in normal (2) and opioid tolerant (1) SH-SY5Y cells were recently described. In investigating the effect of various differentiating agents on these processes, the distinct modulation of μ and δ opioid receptors by RA and TPA, including a contrasting regulation of μ ligand binding and μ receptor coupling by TPA, was observed in cells exposed to these compounds for 9-11 days (Table 1). These results corroborate, in part, the previously described enhancement of AC inhibition by μ opioids in SH-SY5Y cells differentiated with RA (7), and reveal a novel regulation of μ opioid signal transduction by long-term TPA action: the concurrent receptor up-regulation and effector desensitization. In contrast, TPA minimally affected both ligand binding to and effector coupling of the δ opioid receptor.

TPA has been shown to acutely inhibit the coupling to AC of several inhibitory receptors including the δ opioid (8). Thus, it is plausible to implicate inhibitory G proteins, the common transducers of signal transduction in these processes, as the target of TPA action. Indeed, the functional inactivation of G_i by TPA-induced phosphorylation has been described (9) and the involvement of PKC, whose activity is potently enhanced by phorbol esters, in that process was suggested (8,9). While it was shown that differentiation of SH-SY5Y cells by RA did not alter their content of G_i or G_o, the effect of TPA was not investigated in that study (10).

As shown here, our data on the binding of GTPγS and opioid stimulation of low-Km GTPase do not reveal an impairment in the function of G protein in SH-SY5Y cells differentiated by TPA (Table 1). However, these findings might not disclose possible changes in the relevant G protein subtype: the coupling of μ opioid receptors to adenylyl cyclase is preferentially mediated by G_o (4). Furthermore, while the preliminary results of Western blots showed no difference in the content of G_o following differentiation with RA or TPA (not shown), the obtained resolution could be insufficient to reflect

partial covalent modification of this G protein subtype. To elucidate the mechanism by which TPA induces the concurrent up-regulation of μ opioid receptor and desensitization of AC in SH-SY5Y cells, ongoing experiments focus on the relationship between G_o and PKC, whose activators and inhibitors are potent differentiating agents of SH-SY5Y cells (11), and PLC, a G_o-selective effector whose activation stimulates PKC (12). These potential interrelations illustrate the importance of receptor cross-talk, processes by which a given receptor is regulated by others functioning in the same milieu.

Table 1. Parameters of receptor binding, adenylyl cyclase inhibition and low-Km GTPase stimulation by opioids and GTPγS binding. The saturation binding of [^{3}H]DAMGO and [^{3}H]DPDPE in membranes from untreated and differentiated SH-SY5Y cells was resolved by Scatchard analysis (K_d and B_{max}). In other experiments, intact cells were incubated with unlabeled opioids and their content in cAMP determined by radioligand binding. The maximal inhibition of adenylyl cyclase (I_{MAX}) was related to it's activity in the absence of opioids. In separate experiments, membranes were incubated with GT^{32}P in the absence and presence of 10 μM opioid. Liberated ^{32}P, corresponding to low-Km GTPase activity, was quantified by liquid scintillation counting and expressed as stimulation by opioids (S_{MAX}). In addition, the saturable, specific binding of GTPγ^{35}S was resolved by Scatchard analysis into two sites ($K_{d1\&2}$, $B_{max1\&2}$). Shown throughout this table are mean values $\pm$ SEM of 3-5 experiments, carried out in duplicate.

Experiment	Cell treatment	Kd (nM)	B_{max} (fmol/mg prot)	I_{max} (%)	S_{MAX} (%)
DAMGO	None	0.33	70 ± 3	20 ± 3	13 ± 2
	TPA	0.37	210 ± 3	26 ± 3	38 ± 2
	RA	0.45	198 ± 6	73 ± 5	19 ± 3
DPDPE	None	3.4	78 ± 5	19 ± 4	6 ± 3
	TPA	2.4	75 ± 3	18 ± 3	17 ± 6
	RA	2.7	242 ± 13	45 ± 5	15 ± 2

Cell treatment	Kd (nM)		B_{max} (fmol/mg)	
	1	2	1	2
None	0.08	2.88	213 ± 54	261 ± 50
TPA	0.08	1.23	177 ± 26	269 ± 62
RA	0.04	0.65	150 ± 20	194 ± 11

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