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Cholesterol reduction by methyl-β-cyclodextrin attenuates the delta opioid receptor-mediated signaling in neuronal cells but enhances it in non-neuronal cells

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ABSTRACT

Opioid receptors have been shown to be located in and regulated by lipid rafts/caveolae in caveolin-rich non-neuronal cells. Here, we found that caveolin-1 level was very low in rat brain and undetectable in NG108-15 cells, which endogenously express delta opioid receptors (DOR). Rat caudate putamen (CPu) membranes, NG108-15 cells and CHO cells stably transfected with FLAG-mouse-DOR (CHO-FLAG-mDOR) were homogenized, sonicated in a detergent-free 0.5 M Na_2CO_3 buffer and fractionated through discontinuous or continuous sucrose density gradients. About 70% of opioid receptors in CPu and DOR in both cell lines were present in low-density (5–20% sucrose) membrane domains enriched in cholesterol and ganglioside M1 (GM1), characteristics of lipid rafts in plasma membranes. In both cells, stimulation with permeable or non-permeable full agonists, but not with partial or inverse agonists, for 30 min shifted ~25% of DORs out of rafts, by a naloxone-reversible and pertussis toxin-insensitive mechanism, which may undergo internalization. Methyl-β-cyclodextrin (MCD) treatment greatly reduced cholesterol and shifted DOR to higher density fractions and decreased DPDPE affinities. MCD treatment attenuated DPDPE-induced [^{35}S]GTPγS binding in CPu and NG108-15 cells, but enhanced it in CHO-FLAG-mDOR cells. In CHO-FLAG-mDOR cells, $G_{\alpha i}$ co-immunoprecipitated with caveolin-1, which was shown to inhibit $G_{\alpha i/o}$, and MCD treatment dramatically reduced the association leading to disinhibition. Thus, although localization in rafts and agonist-induced shift of DOR are independent of caveolin-1, lipid rafts sustain DOR-mediated signaling in caveolin-deficient neuronal cells, but appear to inhibit it in caveolin-enriched non-neuronal cells. Cholesterol-dependent association of caveolin-1 with and the resulting inhibition of G proteins may be a contributing factor.

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Abbreviations: CPu, caudate putamen; CHO cells, Chinese hamster ovary cells; CHO-FLAG-mDOR, CHO cells stably transfected with FLAG-mDOR cDNA; DPDPE, [D-Pen^2,D-Pen^5]-Enkephalin; DTT, dithiothreitol; FLAG epitope, (DYKDDDDK); FLAG-mDOR, FLAG-tagged mouse δ opioid receptor; GM1, ganglioside M1; GPCRs, G protein-coupled receptors; HRP, horseradish peroxidase; MCD, methyl-β-cyclodextrin; MES, 2-morpholinoethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TBS-T, 10 mM Tris–HCl, 159 mM NaCl, 0.1% Tween-20, pH 7.4

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1. Introduction

At least three types of opioid receptors (μ, δ and κ) mediate pharmacological effects of opioid drugs and physiological actions of endogenous opioid peptides. The δ opioid receptor (DOR) has been associated with analgesia, morphine tolerance and mood regulation [1,2]. The δ opioid agonists may potentially be used as analgesics with less side effects associated with the μ agonists as well as anxiolytics and antidepressants [2,3]. The DOR is mainly distributed in neurons, and is also found in non-neuronal cells, including the rat and human heart myocytes [4,5]. In the heart, activation of DOR produces negative ionotropic effects and δ agonists have cardio-protective effects [6,7]. Opioid receptors are members of the rhodopsin sub-family of G protein-coupled receptors (GPCRs) and are coupled primarily to G_i/G_o proteins to modulate several downstream effectors, including inhibition of adenylyl cyclases, enhancement of K^+ conductance, attenuation in Ca^{2+} conductance and stimulation of p42/p44 mitogen-activated protein (MAP) kinases (for a review, see [8]).

Lipid rafts are small, low-density, cell plasma membrane domains enriched in cholesterol and glycosphingolipids (e.g., GM1) in the outer layer. Recently, it was proposed that they should be termed ‘‘membrane rafts’’, as it has become increasingly apparent that proteins play a major role in their formation and contribute to their function [9]. Thus, the term membrane rafts and lipid rafts will be used interchangeably. Since Brown and Rose [10] gave the operation definition of lipid rafts, the concept has been developed largely based on their biochemical nature of insolubility in non-ionic detergents at low temperature and high buoyancy in density gradients. Lipid rafts are classified into planar lipid rafts and caveolae. Morphological identification of planar lipid rafts has been elusive [11]. One the contrary, electron micrographs show that caveolae are flask-shaped membrane invaginations at plasma membranes in most differentiated cells [12]. Caveolins, three structural and scaffolding proteins, form a cytoplasmic coat on the invaginated structures and appear to stabilize the identifiable shape of caveolae [13].

Of particular interest has been the notion that lipid rafts act as organizational platforms for signal transduction, as a variety of membrane proteins involved in signaling were found to be enriched in or recruited into lipid rafts/caveolae [12,14,15]. Caveolins have been reported to interact with and concentrate many signaling proteins within caveolae, and, in most cases, negatively regulate their activities [12,16]. A number of GPCRs and their downstream effectors, such as G_α proteins, protein kinase C and adenylyl cyclases, have been demonstrated to be regulated by lipid rafts/caveolae [14,15,17].

Investigations on effects of lipids on binding properties and signaling of opioid receptors could be traced back to 1980s. For examples, incorporation of cerebroside sulfate (a glycosphingolipid) or phosphatidylcholine augments both the potencies and the efficacies of morphine and enkephalin to regulate adenylyl cyclase activity in N18TG2 cells without changing the number of the DOR binding sites [18]. Increasing membrane cholesterol in N1E-115 neuroblastoma cells reduced [^{3}H]met-enkephalin binding activity at DOR [19]. Lipids were required for the binding activity of partially purified μ opioid receptors and specificity of the requirement was defined [20].

Opioid receptors, like many other GPCRs, have been recently shown to locate in lipid rafts/caveolae in caveolin-rich non-neuronal cells, and such localization plays important roles in receptor functions, including the κ opioid receptors expressed in CHO cells [21], the μ opioid receptors transfected into HEK293 cells [22] and μ and δ opioid receptors in adult rat cardiac myocytes [23,24]. The μ, δ and κ opioid receptors have caveolin-1-binding consensus sequences (the ‘‘$\phi X \phi XXXX \phi$ motif’’, where ϕ is an aromatic residue [25]), ‘‘YAFLDENF’’, at the junction of TMs7 and C-tails. We have found that caveolin-1 co-immunoprecipitated with FLAG-tagged human κ opioid receptors expressed in CHO cells [21].

Neurons in the brain had been demonstrated to be deficient in caveolin-1 and devoid of caveolae [26]. Although numerous GPCRs are present in neurons in the brain, whether GPCRs, including opioid receptors, are localized in low-density cholesterol- and glycosphingolipids-rich membrane domains (non-caveolae lipid rafts) remains unclear. In addition, little is known about the role of the non-caveolae lipid rafts in regulating GPCRs in neuronal cells or tissues.

In this study, we found the opioid receptors in the rat caudate putamen (CPu), the δ opioid receptor (DOR) endogenously expressed in NG108-15 neuroblastoma x glioma hybrid cell line and FLAG-mouse-DOR expressed in CHO cells (CHO-FLAG-mDOR) were localized in lipid rafts. NG108-15 cells have long been used as an *in vitro* neuron-like model to study opioid receptor properties and signaling. We observed that NG108-15 cells had no detectable caveolin-1 and the rat brain expressed a very low level of caveolin-1, whereas there was abundant caveolin-1 in CHO cells. We examined and compared the role of lipid rafts in opioid receptor functions in the three systems and delineated possible mechanisms underlying the differences.

2. Materials and methods

2.1. Materials

[^{3}H]diprenorphine (58 Ci/mmole) and [^{35}S]guanosine 5-(γ-thio)triphosphate (GTPγS) (1250 Ci/mmole) were purchased from Perkin-Elmer Co. (Boston, MA). Naloxone was a gift from the former DuPont/Merck Co. (Wilmington, DE). DPDPE, deltorphin II and etorphine were provided by Drug Supply System of National Institute on Drug Abuse (NIDA). Sodium carbonate, 2-morpholinoethanesulfonic acid (MES), glycerol, ethylenediamine tetraacetic acid (EDTA), ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), dithiothreitol (DTT), PMSF, GDP, GTPγS, methyl-β-cyclodextrin (MCD), HAT and anti-FLAG monoclonal antibody (M1) were purchased from Sigma Co. (St Louis, MO). For phosphate assay, hydrogen peroxide, Fisk-Subbarrow reducer and phosphate standard were obtained from Sigma (St Louis, MO) and ammonium molybdate was purchased from Fisher (Newark, DE). Ammonium persulfate was purchased from Bio-Rad Laboratories (Hercules, CA). Anti-GM1 polyclonal antibody was purchased from Calbiochem (San Diego, CA). Anti-caveolin-1 monoclonal antibody (clone 2297) and anti-flotillin-1 monoclonal antibody were obtained from BD Transduction Laboratories (San Jose, CA). Polyclonal anti-$G_{\alpha i3}$ antibody,

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