STABILIZATION OF HIGH-AFFINITY OPIOID AGONIST BINDING IN NEURAL CELL MEMBRANES RIGIDIFIED BY CHOLESTEROL

Emmerson PJ and Medzihradsky F, Departments of Pharmacology and Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI 48109, U.S.A.

ABSTRACT

Incorporation of cholesteryl hemisuccinate (CHS) into membranes from SH-SY5Y human neural cells increased membrane microviscosity at both surface and core. In the rigidified membranes, μ-selective opioid agonists bound to a high-affinity state of the receptor similar to that observed upon receptor-G protein coupling. However, the high affinity state in rigidified membranes was unaltered by guanine nucleotides and pertussis toxin (PTX), and was not regulated by sodium. In contrast, the binding of opioid antagonists was insensitive to the membrane modification.

METHODS

The culture of SH-SY5Y human neural cells, ligand binding, and the isolation of cell membranes were carried out essentially as described (1). Cells lysed in hypotonic phosphate were centrifuged 10 minutes at 500g and resuspended in a Dounce homogenizer three times to remove the nuclear pellet. Membrane modification with CHS and measurement of microviscosity were implemented as reported earlier (2). For the determination of cholesterol and incorporated CHS, cell membranes with and without addition of appropriate standards were incubated at room temperature with 2.5 N KOH for 30 minutes. After neutralization, total cholesterol was quantified by an enzymatic-fluorometric procedure (3). Recovery of CHS was greater than 95%.

RESULTS AND DISCUSSION

Regulation of ligand binding to G protein-coupled receptors, including the opioid, occurs through receptor-transducer association and dissociation, a process modulated by guanine nucleotides and ions. The high-affinity binding state, representing the ternary complex of agonist-occupied receptor and G protein (4), converts in the presence of GTP or its stable analogues into the low-affinity, G protein-uncoupled, form of the receptor. Previous studies with several receptors, including α1- and ß-adrenergic and dopamine have described the formation of a high-affinity agonist state of receptor at low (2-4 °C) temperatures (5-7) or by a combination of deoxycholate and low temperature (8). Initial inactivation of G protein (6,8) or the presence of guanine nucleotides (5,7,8) had no influence on the high-affinity agonist binding state, observed in membranes of various cells. No common mechanism was forwarded to account for the formation and locking of the high-affinity receptor state observed in these studies.

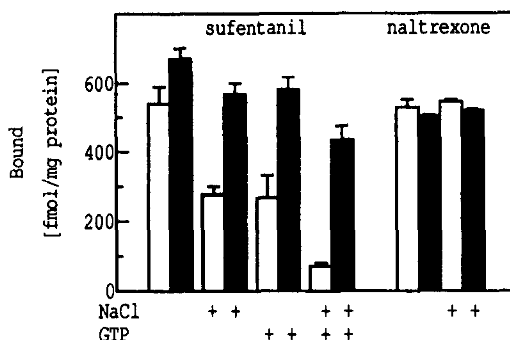

FIG. 1. Sufentanil and naltrexone binding in rigidified membranes. Specific binding of 0.1 nM [3H]sufentanil or 0.5 nM [3H]naltrexone to control membranes (open bars) or membranes modified with 2 μmol/mg protein CHS (closed bars) was determined in the absence and presence of 150 mM NaCl and 100 μM GTPγS. Shown are the mean values +/- SEM of 3-5 experiments carried out in duplicate.

As shown previously, membrane rigidification (by incorporation of cholesterol) or fluidization (by incorporation of cis-fatty acids) potently affected ligand binding to (9) and the conversion between agonist- and antagonist-favoring states of the μ opioid receptor in rat brain membranes (2). These

studies have implicated membrane microviscosity in the mechanism underlying the observed modulation of opioid receptor function. In the present study, we have examined the influence of membrane rigidification on different affinity states of the μ opioid receptor. Rigidification of SH-SY5Y cell membranes by the incorporation of 0.5 - 2 μmol / mg protein CHS selectively enhanced the high-affinity binding of μ opioid agonists. As shown with [3H]sufentanil, the membrane effect was specific for agonists: the binding of [3H]naltrexone was unaffected by the rigidification (Fig. 1). While in unmodified membranes the high-affinity binding of sufentanil was attenuated by sodium and GTPγS, membrane rigidification resulted in high-affinity sufentanil binding that was devoid of regulation by ions and nucleotides (Fig. 1). Furthermore, the down-regulation of high-affinity agonist binding ([3H]DAMGO and [3H]sufentanil) in membranes of cells exposed to pertussis toxin was reversed by subsequent incorporation of CHS, indicating a G protein independent mechanism for the stabilization of this state of the receptor in the rigidified membranes (Fig. 2).

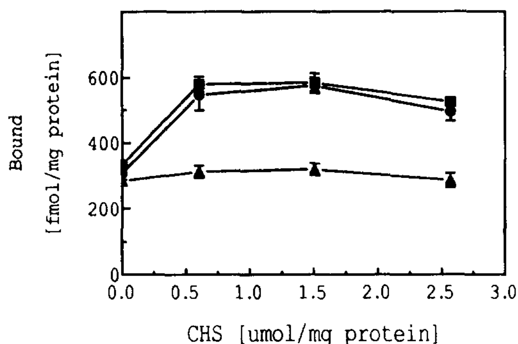

FIG. 2. Opioid agonist and antagonist binding in membranes of pertussis toxin-treated cells. Cells were treated for 24 hours with 50 ng/ml PTX. Membranes were collected as described in Methods, and divided in two batches: one was used as such, the other modified by the incorporation of CHS. Subsequently, the specific binding of 0.1 nM [3H]sufentanil, 1 nM [3H]DAMGO, and 0.5 nM [3H]naltrexone was determined. Shown are the mean values +/- SEM of 3 experiments carried out in duplicate.

Considering the similarity of the phenomena observed in the modified SH-SY5Y cells and those described earlier for receptor binding at low temperatures (5,6), it is tempting to implicate membrane microviscosity as the contributing factor in the latter studies as well. Howsoever, the stabilization of high-affinity opioid ligand binding following CHS-induced rigidification of neural cell membranes, a process shown to occur in aging or as a consequence of pathophysiological changes (10), represents a potentially novel regulatory mechanism of membrane signal transduction functioning in addition to that dependent on G proteins. In ongoing experiments, the cellular function of opioid receptor in rigidified membranes (ability to couple to G protein and adenylyl cyclase), and the direct interaction of receptor with the incorporated sterol in the lipid boundary layer are being investigated.

ACKNOWLEDGEMENT
This work was supported in part by USPHS grant DA 04087 and travel funds from the International Narcotic Research Conference (PJE).

REFERENCES:
1. B.D. Carter and F. Medzihradsky (1992) J. Neurochem. 58, 1611-1619.
2. D. Lazar and F. Medzihradsky (1993) J. Neurochem. 61 (in press).
3. J.G. Heider and R.L. Boyet (1978) J. Lipid Res. 19, 514-518.
4. A.E. Remmers and F. Medzihradsky (1991) Proc. Nat. Acad. Sci. USA 88, 2171-2175.
5. C.J. Lynch, R. Charest, P.F. Blackmore and J.H. Exton (1985) J. Biol. Chem. 260, 1593-1600.
6. C.J. Lynch, S.J. Taylor, J.A. Smith and J.H. Exton (1988) FEBS Lett. 229, 54-58.
7. M. Watanabe, S.R. George and P. Seeman (1985) Biochem. Pharmacol. 34, 2459-2463.
8. G. Neufeld, S. Steiner, M. Korner and M. Schramm (1983) Proc. Nat. Acad. Sci. USA 80, 6441-6445.
9. D. Lazar and F. Medzihradsky (1992) J. Neurochem. 59, 1233-1240.
10. M. Shinitzky (1984) Physiology of Membrane Fluidity, Vol 1, CRC Press, Boca Raton, FL.