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Fluidity of detergent micelles plays an important role in muscarinic receptor solubilization

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Abstract. In order to find a suitable reagent for extracting the muscarinic receptor from rat brain membranes 14 different detergents were tested. Only the plant glycoside digitonin efficiently solubilized the receptor protein in its native form. At the same time microviscosity of detergent micelles was determined by measuring the fluorescence polarization of a hydrophobic fluorescent probe diphenylhexatriene incorporated into the micelles. In the case of digitonin the polarization value was close to the corresponding value obtained for rat brain membrane fragments, while for the other detergents studied it remained considerably lower. The results obtained indicate that the fluidity of detergent micelles may play an important role in retaining the active conformation of the solubilized muscarinic receptor.

Keywords. Muscarinic receptor; receptor solubilization; diphenylhexatriene; fluoresence polarization; micelle fluidity.

Introduction

The muscarinic acetylcholine, receptor is located in synaptic membranes of the central nervous system and several peripheral tissues (Snyder *et al*., 1975). Solubilization of this integral membrane protein is an important step in the procedure of its purification and molecular characterization. However, there seems to be a very limited number of solubilizing agents able to extract the muscarinic receptor from biomembranes without a considerable loss of its activity (Aronstam *et al*., 1978; Sokolovsky, 1984). The best results so far have been obtained using the plant glycoside digitonin (Rinken *et al*., 1987; Raidaru *et al*., 1989) but the physico-chemical basis of its action is not known. Therefore it is important to evaluate the properties of detergents essential for their ability to solubilize the receptor protein retaining its ability to specifically bind ligands. In the present study we compare some physico-chemical characteristics of detergent micelles, critical miceller concentration (CMC), aggregation number and fluidity with the ability of these agents to solubilize the native receptor.

Materials and methods

Suspension of rat brain membranes in 50 mM K-phosphate buffer (pH 7·4) was prepared as described by Langel *et al*. (1982).

Solubilization of the native receptor was carried out as follows: suspension of rat brain membrane fragments (approximately 1 mg protein/ml) was incubated with detergent solutions of different concentrations for 30 min at 0°C and centrifuged at

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Abbreviations used: CMC, Critical micellar concentration; QNB quinuclidinyl benzilate; DPH, diphenylhexatriene; CHAPS, 3-[(3-cholamidopzopyl)-dimethylammonio]-l-propanesulfonate; SDS, sodium dodecyl sulphate.

100,000 g for 1 h. Components remaining in the supernatant were considered solubilized.

The amount of solubilized receptor was determined by specific binding of 3 nM [^{3}H] quinuclidinyl benzilate (QNB) (43Ci/mmol, Amersham) at 25°C. Receptor-QNB complex was separated from the excess of free ligand on Sephadex G-50 columns in the case of solubilized receptor and on GF/B glass-fibre filters (Whatman) in the case of membrane-bound receptor.

In order to solubilize the receptor-ligand complex the membrane fragments were incubated with 3 nM [^{3}H]QNB for 30 min prior to adding the detergent solution.

Non-specific binding of [^{3}H]QNB was determined in the presence of 10 μM atropine sulphate (Merck).

Protein concentration was determined by the modified method of Lowry (Peterson, 1977) using bovine serum albumine as standard.

Microviscosity of detergent micelles was estimated by means of measuring the fluorescence polarization of a hydrophobic fluorescent probe diphenylhexatriene (DPH) incorporated into the micelles. Two μl of DPH dissolved in tetrahydrofuran was added into a detergent solution to achieve a final concentration of the probe of 1 μM. The mixture was incubated for 30 min at room temperature and the fluorescence polarization of DPH was measured on Hitachi-850 spectro-fluorimeter at excitation and emission wavelengths of 358 and 430 nm, respectively. In all cases, detergent concentrations well above their CMCs were used. Digitonin was obtained from Merck, 3-[(3-cholamidopzopyl)-dimethylammonio]-1-propanesulfonate (CHAPS) from Calbiochem and other detergents from Sigma.

Results and discussion

Out of the 14. detergents, studied only the plant glycoside digitonin was able to effectively solubilize the native muscarinic receptor with an yield of 34%. Besides digitonin, CHAPS was also able to solubilize the native receptor to a certain extent, the maximal yields reaching only 6–8%. In the case of sodium dodecyl sulphate (SDS), cholates and detergents of Triton X and Tween series no muscarinic ligand binding activity could be detected in the solution although they were able to extract major part of the protein present in the membrane (table 1).

As both digitonin and CHAPS contain the steroid core, its presence in the detergent molecule can be considered important in retaining the active conformation of the muscarinic receptor. However, the presence of this structural element is not sufficiency as cholate and deoxycholate turned out to be ineffective. In the latter cases the ionic charge situated near the steroid core probably facilitates the denaturation of the receptor by these detergents while CHAPS, being a zwitterionic molecule, where the ionic charges are separated from the steroid core by several methylene groups, is a much weaker denaturing agent allowing the solubilization of the muscarinic receptor at low detergent concentrations.

In addition to digitonin and CHAPS the receptor-QNB complex could also be solubilized with Triton X-100, X-102, X-114, X-165 and SDS (table 1). In all these cases the complex solubilization yields did not exceed 50% of its total amount in the membrane. This value could not be increased using mixtures of the studied detergents (data not shown). At the same time it was shown that after detergent treatment no receptor-QNB complex could be detected in the membrane fraction.

Table 1. Muscarinic receptor solubilization yields and micelle-characterizing parametres for some detergents.

Detergent	CMC (mM)*	Aggregation number*	Fluorescence polarization of DPH	% Solubilization of native receptor	% Solubilization of receptor [^{3}H] QNB complex	% Solubilization of protein yield**
Digitonin	0·16	60	0·359 ± 0·004	34 ± 4	41 ± 5	70 (0·6%)
CHAPS	8–10	4–14	0·167 ± 0·003	6 ± 1	8 ± 2	–
Sodium cholate	13–15	2–4	0·102 ± 0·005	0	0	50 (1%)
Sodium deoxycholate	4–6	4–10	0·132 ± 0·004	0	0	100 (1%)
Triton X-45	0·11	–	–	0	0	–
Triton X-100	0·24	140	0·153 ± 0·004	0	50 ± 6	50 (0·3%)
Triton X-102	0·3–0·4	–	–	0	45 ± 3	40 (0·3%)
Triton X-114	0·2	–	–	0	30 ± 4	65 (0·5%)
Triton X-165	0·43	–	0·164 ± 0·004	0	47 ± 2	90 (0·5%)
Triton X-305	–	–	0·167 ± 0·002	0	0	–
Tween 20	0·059	–	–	0	0	35 (1%)
Tween 40	0·029	–	–	0	0	–
Tween 60	0·027	–	–	0	0	–
Tween 80	0·012	58	0·114 ± 0·002	0	0	–
SDS	8·2	62	0·094 ± 0·004	0	50 ± 5	60 (0·03%)

*Data are taken from Helenius and Simons (1975), Chattopadhyay and London (1984) and *A guide to the properties and uses of detergents in biology and biochemistry* (San Diego: Calbiochem) (1987).
**Data correspond to the detergent concentrations (shown in parentheses) giving the highest receptor solubilization yield.

Consequently, about half of the complex dissociates during the solubilization. As in the absence of detergents the receptor-QNB complex dissociation process is very slow (Sokolovsky, 1984), its loss is caused by denaturation of the receptor protein.

Examining the CMCs and aggregation numbers of the detergents used, no relationship between their values and the ability to solubilize the muscarinic receptor could be observed. Thus, these parameters of detergents are not essential for receptor solubilization.

Determination of the fluorescence polarization values of several fluorescent probes, most often DPH, have been used to monitor the fluidity of the probe environment (Shinitzky and Barenholz, 1974: Masturzo *et al*., 1985). In the present study DPH was used for this purpose. Fluorescence polarization values were measured for DPH incorporated into detergent micelles as well as for DPH incorporated into the native membrane containing the muscarinic receptor. In the case of digitonin the polarization value obtained was close to the corresponding value for the membrane (0·28–0·30): for all other studied detergents it remained considerably lower (table 1). Consequently, fluidity of native receptor microenvironment seems to be important for supporting its active conformation in solution. The receptor-QNB complex can also be solubilized with the help of some detergents with low micelle fluidities. As ligands bound to the receptor protein are known to increase its stability (Peterson and Schimerlik, 1984), the complex is probably less sensitive to the physical parametres of its surroundings than the native receptor.

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