ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS
Vol. 258, No. 1, October, pp. 33-41 1987

Thermal Perturbation Studies of Membrane-Bound Acetylcholine Receptor from *Torpedo:* Effects of Cholinergic Ligands and Membrane Perturbants

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Received May 4, 1987

Thermal perturbation techniques have been used to probe structural features of the nicotinic acetylcholine receptor (AcChR). The information obtained from differential scanning calorimetry (DSC) of AcChR membranes (M. C. Farach and M. Martinez-Carrion (1983) *J. Biol. Chem.* **258,** 4176) in the absence and in the presence of cholinergic ligands and local anesthetics, is comparable to that obtained from a simpler technique of heat inactivation of the α-bungarotoxin (α-Bgt) binding sites on the AcChR protein in similar samples. When AcChR membranes are heated at ~1°C/min, heat inactivation of toxin binding sites has a characteristic T_{50} value (temperature at which 50% of the initial capacity to bind α-Bgt remains) of ~60°C. When heated at a constant temperature during increasing periods of time, the rate at which heat inactivation occurs is also characteristic of the temperature chosen for the experiment. The above thermal parameters are also sensitive to perturbation of the AcChR membrane matrix by the presence of subsolubilizing concentrations of detergents. Moreover, elimination of detergents by dialysis allows us to evaluate the reversibility or irreversibility of AcChR thermal destabilization induced by detergents or other membrane perturbants. Under the experimental conditions used, structural destabilization induced by octylglucoside or cholate can be fully reversed by detergent dialysis, while that exerted by deoxycholate cannot. "Thermal gel" analysis of the aggregation of AcChR subunits induced by heat (G. Soler, J. R. Mattingly, and M. Martinez-Carrion (1984) *Biochemistry* **23,** 4630) has also been used to assess the effects of detergent presence on the AcChR protein. When deoxycholate is used as the perturbing agent, there is a particularly effective sulfhydryl-mediated aggregation of the γ-δ subunit group, which appears to correlate with the irreversible destabilization of α-Bgt binding sites induced by that detergent.

The nicotinic acetylcholine receptor (AcChR)[2] is a pentameric transmembrane glycoprotein composed of four different polypeptide subunits (α, β, γ, and δ) in a 2:1:1:1 stoichiometry (for review, see Refs. (1-4). *In vivo,* these complexes appear to exist as dimers linked by a disulfide bond between adjacent δ subunits (5, 6). Binding of cholinergic agonists to extracellular domains on the AcChR protein elicits the formation of a transient cation channel responsible for the initiation of postsynaptic membrane depolarization. Membrane fractions highly enriched in AcChR can be prepared in large amounts from the electric organ of *Torpedo* or *Electrophorus* species.

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[2] Abbreviations used: AcChR, acetylcholine receptor; DSC, differential scanning calorimetry; α-Bgt, α-bungarotoxin; OG, β-D-octylglucoside; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

0003-9861/87 $3.00

Also, the AcChR can be reconstituted into well-defined liposomes, thus providing a good model for studying the lipid dependence of this integral membrane protein (7–9).

The availability of rapid kinetics techniques (10–16) and single channel recordings (patch clamp) (17–20) allows for precise quantitation of AcChR functionality concerning agonist-induced cation translocation. However, the study of structure/function relationships also requires monitoring of structural parameters on a routine basis and this, for membrane proteins, is hindered by the inherent qualities of biological membranes. for instance, the application of spectroscopic techniques such as circular dichroism or Raman (21–23) is usually restricted because of excessive membrane scattering or lipid contributions; thus we are mostly limited to study membrane proteins in a detergent-solubilized form or in reconstituted systems containing a single lipid component. Under these conditions, it is uncertain whether the protein of interest maintains those features exhibited in its native membrane environment. Recently, a differential scanning calorimetry (DSC) study of AcChR was reported (24) in which the native, membrane-bound AcChR exhibited a characteristic thermal transition at 59–60°C which was assigned to irreversible AcChR denaturation and to a complete loss of AcChR function, including binding of α-neurotoxins. Also, the observed endotherm was sensitive to the presence of cholinergic ligands, thus supporting its assignment to specific structural alteration of the AcChR protein.

In this paper, we have used thermal perturbation techniques, including DSC, to probe the AcChR structure. Our results suggest that there is a similarity between the information obtained from a simple technique of heat inactivation of the α-neurotoxin binding sites, and that gathered through the more laborious DSC procedures. The combined use of thermal perturbation techniques allows us (i) to confirm the notion that structural changes in the AcChR protein occur as a consequence of binding of cholinergic agonists under conditions known to cause AcChR desensitization, (ii) to study the destabilization of the AcChR protein induced by the presence of membrane perturbants, and (iii) to begin the characterization of different stages in the complex process of thermal denaturation of AcChR.

MATERIALS AND METHODS

Live *Torpedo marmorata* were obtained through local fishermen during the winter months. The electric tissue was rapidly excised and maintained frozen in liquid nitrogen. Electroplax membranes highly enriched in AcChR were prepared through a procedure which included alkaline extraction of peripheral membrane proteins (25) as previously described (26). The resulting AcChR membranes were finally suspended in a 10 mM Hepes buffer, pH 7.4, containing 100 mM $NaNO_3$, and had specific activities of 30–35 μg of α-bungarotoxin (α-Bgt) bound/mg of membrane protein. Protein concentration was determined as described by Lowry *et al.* (27) and ^{125}I-α-Bgt binding was measured by using a DEAE-cellulose filter disk assay (28). Radiolabeled α-Bgt was either purchased from New England Nuclear or prepared by iodination (Enzymobead reagent, Bio-Rad) of our purified toxin (29).

"Heat inactivation" refers to monitoring the characteristic loss of α-Bgt binding by AcChR membranes as heat is supplied to the samples. Series of 50-μl aliquots of AcChR membranes (~2 mg of protein/ml) were heated in an immersion water bath at a linear rate of ~1°C/min. Temperature was measured using a thermometer (0.1°C accuracy) positioned with a geometry similar to that of AcChR samples. Aliquots were removed at selected temperatures, diluted with an equal volume of ice-cold buffer containing 2% Triton X-100, placed on ice for a minimum of 1 h, then submitted to α-Bgt binding analysis. Alternatively, AcChR membrane samples were heated at a constant temperature for selected periods of time (usually, 1 to 40 min).

"Thermal gel" analysis refers to the procedure described by Lysko *et al.* (30) for the analysis by SDS–PAGE of thermally perturbed proteins. This procedure has been recently used in studies of *Torpedo* AcChR (31). AcChR membrane samples for thermal gel analysis were heated as described above except that, following removal of the heated samples from the water bath, an equal volume of electrophoresis sample buffer without mercaptoethanol was added. The solubilized mixtures were incubated for 30 min at room temperature, prior to electrophoresis in a slab gel with a 5–15% acrylamide exponential gradient and a 3% stacking gel. Electrophoretic conditions and reagents were those of Laemmli (32).

DSC was performed on a Microcal MC1 microcalo-

rimeter. Differences in the heat capacities between 1-ml aliquots of AcChR membranes at 6–10 mg of protein/ml (contained in the "sample" cell of the calorimeter) and buffer alone ("reference" cell) were obtained by raising the temperature at a linear rate of ~1°C/min. (24). Reported transition temperatures (T_D) correspond with those at which there is a maximum differential heat capacity as observed in the thermograms.

The kinetics of passive permeation of monovalent cations (Tl^+) through AcChR membranes was determined, in the absence of added cholinergic agonists, by a "stopped flow/fluorescence quenching" spectroscopic procedure (11, 26, 29, 33). This procedure is based on the fluorescence quenching of an intravesicularly entrapped fluorophore (1,3,6,8-pyrenetetrasulfonate) by externally added cation quencher (Tl^+). As Tl^+ diffuses into the membrane vesicles, collisional quenching with the entrapped fluorophore, which is monitored as a time-dependent decrease of the fluorescence, occurs.

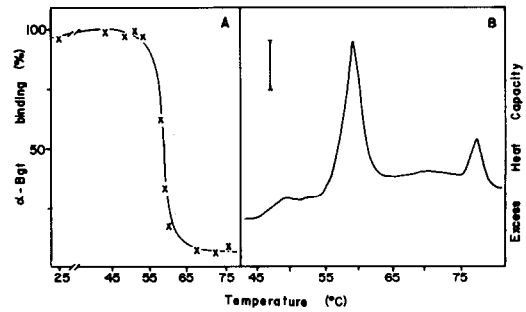

FIG. 1. (A) Effect of controlled heating on the capacity of AcChR membranes to bind α-Bgt. Aliquots of AcChR membranes at ~2 mg of protein/ml were processed as indicated under Materials and Methods and α-Bgt binding was determined. Data are given as percentage of α-Bgt binding with respect to that exhibited by unheated (control) AcChR membranes. (B) DSC thermogram of alkaline-extracted AcChR membranes at ~10 mg of protein/ml. Experimental conditions are given under Materials and Methods. Bar indicates 0.16 mcal × $°C^{-1}$.

RESULTS

(1) Heat Inactivation and Differential Scanning Calorimetry of Membrane-Bound AcChR

(A) Effect of cholinergic ligands and local anesthetics. When alkaline-extracted AcChR membranes from *Torpedo* are submitted to the heat inactivation procedure, there is a temperature-dependent loss of the capacity to bind α-Bgt (Fig. 1A). The process starts at 54–55°C and reaches completion at about 67°C where remaining levels of α-Bgt are 5–10% of the total initially present in unheated AcChR membranes. Heat inactivation of α-Bgt binding sites follows a sigmoid-like pattern with a characteristic T_{50} value (temperature at which 50% of α-Bgt binding remains) at about 59–60°C. Likewise, DSC of AcChR membranes at scan rates similar to those used in the heat inactivation assays (~1°C/min.), shows a main thermally induced event which exhibits a transition temperature of 59–60°C (Fig. 1B and Ref. (24)).

Heat inactivation studies conducted in the presence of cholinergic ligands (Table I) show effects which resemble those obtained from DSC studies in similarly perturbed membranes: The presence of carbamylcholine and D-tubocurarine produces an increase and no effect, respectively, in the T_{50}'s of the curves corresponding to thermally induced loss of α-Bgt binding to AcChR membranes. Because of the time scale of either the heat inactivation or the DSC procedures, the observed effects of carbamylcholine should reflect structural changes related to AcChR desensitization caused by prolonged exposure to the cholinergic agonist. Our observation on a more stable protein structure (higher T_{50} or T_D) induced by the presence of carbamylcholine is consistent with a recent suggestion that the desensitized state represents a low free energy form of AcChR (34).

Heat inactivation studies similar to those described above were carried out in the presence of local anesthetics (tetracaine and mepivacaine at concentrations up to 10^{-2} M) (Table I). No differences were found with respect to untreated AcChR membrane samples. This seems to be consistent with the lack of effects reported for local anesthetics in previous DSC studies (24).

(B) Effect of detergents. Heat inactivation studies of AcChR membranes conducted in the presence of detergents at concentrations below those required to produce membrane solubilization, are shown in Fig. 2. For the three detergents

TABLE I

EFFECTS OF CHOLINERGIC LIGANDS AND LOCAL ANESTHETICS ON HEAT INACTIVATION OF TOXIN BINDING SITES AND DSC TRANSITION TEMPERATURES OF MEMBRANE-BOUND AcChR

Compound added	T_{50} (°C)[a]	T_D (°C)[b]
None	60	59.1
Carbamylcholine		
10^{-5} M	63	—
10^{-4} M	—	61.6
10^{-3} M	63	—
D-Tubocurarine		
10^{-5} M	—	58.7
10^{-3} M	60	—
Tetracaine		
10^{-3} M	60	59.1
10^{-2} M	60	—
Mepivacaine		
10^{-3} M	60	—

[a] Reported T_{50} values are the average from three to six different experiments. Standard deviation of heat inactivation data is within ±0.3°C, which is larger than that corresponding to DSC data. Nevertheless, the heat inactivation procedure can easily distinguish between differences in T_{50} values of 1°C or higher.

[b] DSC data taken from Farach and Martinez-Carrion (24).

used, increasing the detergent concentration produces an increased heat sensitivity of the α-Bgt bindng sites. Thermal destabilization induced by detergents causes a decrease in T_{50} values and also increases the temperature range during which heat inactivation of toxin binding sites occurs.

A comparison of the destabilizing effects caused by equal concentrations of each detergent reveals that there is a less pronounced increase in heat sensitivity of the α-Bgt binding sites when cholate is used as the perturbing agent. OG is somewhat more drastic than cholate, while deoxycholate is much more effective than either cholate or OG in destabilizing the α-neurotoxin binding sites. Differences observed for the detergents used do not appear to correlate with their critical micellar concentrations, which are approximately 20, 14, and 5 mM for OG, cholate, and deoxycholate, respectively (35, 36).

DSC of AcChR membranes in the presence of OG (Fig. 3), reveals that all endotherms in the thermogram occur at lower temperatures and become increasingly flat and wider as the detergent concentration increases. It should be noted that while the presence of detergent affects all DSC transitions, the presence of cholinergic agonists was reported to affect only the 59–60°C endotherm specifically assigned to alteration of AcChR structure (24).

The effects of low OG concentrations on passive membrane permeability are shown in Fig. 4. The apparent rate constant for passive permeation of Tl^+ increases more

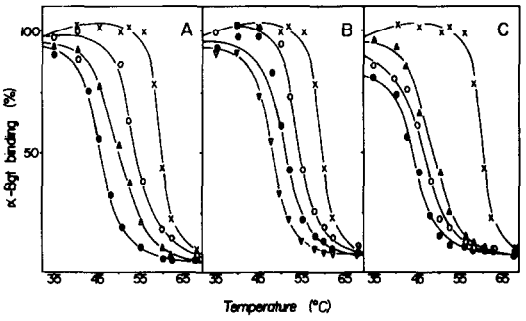

FIG. 2. Heat inactivation of α-Bgt binding sites in AcChR membranes preincubated overnight, at 4°C, in the presence of OG (A), sodium cholate (B), and sodium deoxycholate (C), at concentrations of zero (control) (X), 5 (▲), 10 (○), 15 (△), 20 (●), or 50 (▽) mM. Data are given as the percentage of α-Bgt binding with respect to unheated AcChR membranes, in the absence of added perturbants.

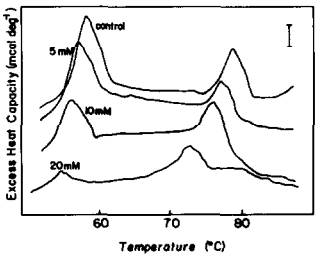

FIG. 3. DSC thermograms of AcChR membranes (~7 mg of protein/ml) in the presence of the indicated concentrations of OG. Small aliquots from a concentrated OG solution were added to identical 1.2/ml aliquots of AcChR membranes. The time lag between OG addition and the beginning of the thermal scan was approximately 2–3 h. Bar indicates 0.16 mcal × °C^{-1}.

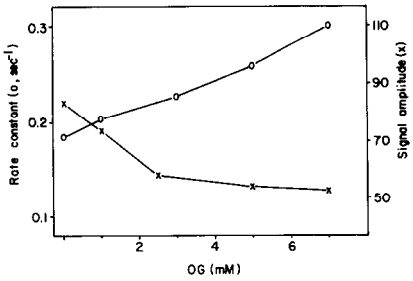

FIG. 4. Effects of subsolubilizing concentrations of OG on the apparent rate constant for passive Tl^+ influx (O) through AcChR membranes. Several 25-s traces were recorded for each experiment and computer fitted to a Stern-Volmer equation containing a time-dependent quencher concentration term (10), to obtain apparent rate constant values for the passive cation influx process. The average values from such determinations have been plotted. Also, leakage of the intravesicularly entrapped fluorophore used in the stopped-flow procedure to measured Tl^+ influx (see Materials and Methods), is illustrated by the decrease in the amplitude of the fluoresence signal (X, relative fluorescence units) caused by increasing concentrations of OG. Temperature was 22°C.

than twofold when AcChR membranes are pretreated with OG at concentrations ranging from 0 to 7 mM. At these low concentrations, elimination of the detergent by dialysis results in apparent rate constants for both passive permeability and AcChR-mediated Tl^+ influx, which are undistinguishable from those exhibited by untreated membranes (11, 29).

Detergent dialysis is also used in Fig. 5 to illustrate the ability of the detergent-treated AcChR to regain those thermal features (T_{50}'s) characteristic of untreated samples. Depending upon the initial concentration of added detergent, a full recovery of thermal stability can be observed for cholate and OG-treated samples. However, thermal destabilization caused by deoxycholate, at any of the detergent concentrations attempted, cannot be fully reversed upon detergent dialysis.

Thermal destabilization of α-Bgt binding sites by the presence of detergents can also be assessed through heating experiments in which AcChR samples are exposed to a constant temperature for different periods of time (Fig. 6). Loss of α-Bgt binding to control AcChR membranes under these conditions, appears to follow a single exponential decay. Half times ($t_{1/2}$) for such decays at a given temperature have a constant value characteristic of unperturbed AcChR membranes (see legend to Fig. 6). The presence of detergents increases the rate of thermal inactivation of α-Bgt binding sites in a concentration-dependent manner. When this heating procedure is used to compare the destabilizing effects of equal concentrations of the different detergents (Fig. 6), it is also observed that OG and cholate, respectively, are milder perturbants than deoxycholate.

(2) Thermal Gel Analysis of AcChR Subunit Aggregation

Figure 7 illustrates a thermal gel analysis of heated AcChR membranes. Progressive disappearance of electrophoretic bands is due to the heat-induced, sulfhydryl-mediated aggregation of AcChR subunits (31). Densitometry scans from six to eight different SDS-PAGE slabs similar to that shown in Fig. 7, were averaged and used to produce the data in Figs. 8A and 8B. The standard deviation of densitometry data was approximately 10%. Figure 8A shows the results obtained from AcChR

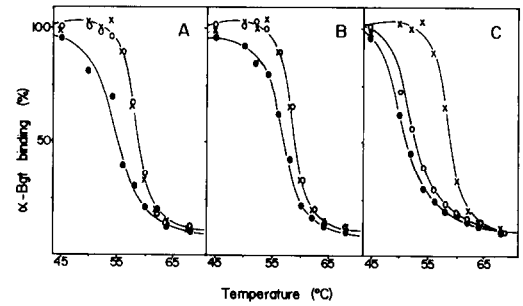

FIG. 5. Reversibility of detergent effects on heat inactivation of α-Bgt binding sites. Data are given as in Fig. 2. AcChR membrane samples were incubated overnight at 4°C, in the presence of OG (A), sodium cholate (B), and sodium deoxycholate (C), at concentrations of zero (X), 10 (O), and 20 (●) mM. The detergent/membrane incubation mixtures were extensively dialyzed (5 × 500 ml changes during a 48-h period) and submitted to the heat inactivation procedure as described under Materials and Methods.

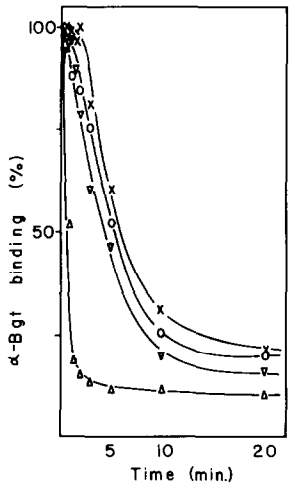

FIG. 6. Time-dependent effect of heating at a constant temperature, on heat inactivation of α-Bgt binding sites. At 60°C, an averaged $t_{1/2}$ value of ~10 min was obtained by computer fitting experimental data from six different "control" membrane preparations (X), to a single exponential. The effect of detergents in increasing the rate of inactivation of α-Bgt binding sites at 60°C, is illustrated for OG (○), sodium cholate (▽), and sodium deoxycholate (△), at a final concentration of 5 mM. Additional information on the rates of inactivation of α-Bgt binding sites at different temperatures and detergent concentrations, is available from the authors upon request.

samples heated under conditions identical to those used in Fig. 1A. Irreversible aggregation of AcChR subunits, as shown in Fig. 8A, exhibits a much lower degree of cooperativity (occurs within a wider temperature range) than that observed for heat inactivation of α-Bgt binding sites. Figure 8B illustrates aggregation of polypeptide subunits from AcChR samples which have been heated at 60°C for increasing periods of time. The disappearance of AcChR subunits from the electrophoretic profile occurs at a higher rate for the γ and δ subunits in comparison to that observed for the α and β subunits (31). This behavior seems to be consistent with the observation that the degree of homology of the α-β and γ-δ pairs is higher than between any other combination of AcChR subunits (37). A comparison of results from Figs. 8B and 6, also suggests that inactivation of α-Bgt binding sites under those conditions is faster than aggregation of α and β subunits, but slower than that observed for the γ and δ subunits.

Results from thermal gel experiments conducted on AcChR samples in the presence of detergents are shown in Fig. 9. The rates of loss of all four AcChR electrophoretic bands are increased with relation to the control. Again, cholate and OG cause a similar effect on the observed electrophoretic patterns. Deoxycholate however, is particularly effective in causing a more rapid disappearance of the γ-δ subunits, which became almost undetectable after 5 min of exposure at 60°C.

DISCUSSION

Finding techniques to probe the structural features of native, membrane-bound

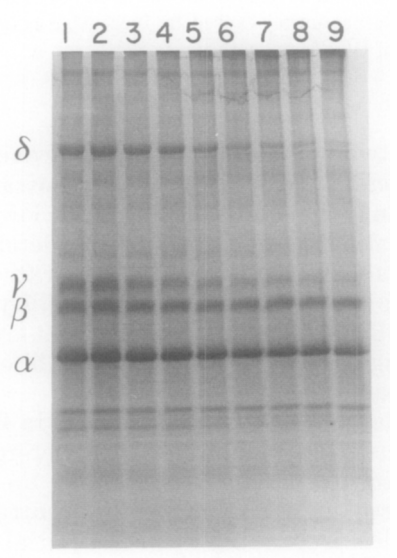

FIG. 7. Representative thermal gel analysis of the aggregation of AcChR subunits. For the example shown, aliquots of AcChR membranes were heated at a constant temperature of 60°C during 0 (lanes 1 and 2), 1 (lane 3), 2 (lane 4), 5 (lane 5), 10 (lane 6), 15 (lane 7), 20 (lane 8), and 40 (lane 9) min, and processed as described under Materials and Methods. Coomassie blue stained gels, or their photographic negatives, were scanned in either a Pherogram Densidig densitometer or a Beckman DU-8 spectrophotometer to obtain relative absorbance data such as presented in Figs. 8 and 9. Apparent molecular weights for AcChR subunits were: α, 40,000; β, 50,000; γ, 60,000, and δ dimers, 130,000.

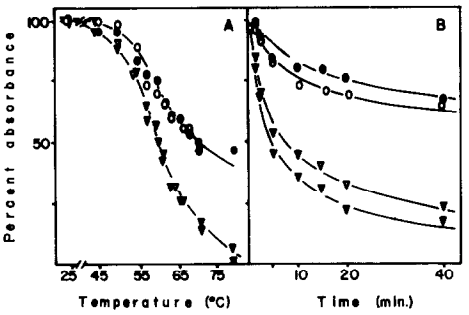

FIG. 8. Effect of controlled heating on the SDS-PAGE profiles of AcChR membranes under nonreducing conditions. AcChR membranes (~2 mg of protein/ml) were (A) heated at the indicated temperatures, at a linear rate of 0.8–0.9°C/min, or (B) exposed at a constant temperature of 60°C for the indicated periods of time, and processed as described under Materials and Methods. Symbols ○, ●, ▽, and ▼, are used to indicate relative intensities of electrophoretic bands corresponding to α, β, γ, and δ subunits, respectively. Computer fitting of experimental data from B to single exponentials, yielded estimates for the half-times ($t_{1/2}$) of aggregation of AcChR subunits which were 19.6, 11.4, 6.3, and 5.8 min, for the α, β, γ, and δ subunits, respectively.

AcChR in relation to its function constitutes a challenge for researchers in this field. Similar to a previously published DSC approach (24), heat inactivation of α-Bgt binding sites (i) provides specific monitoring of AcChR structural features since it is based on binding of highly specific neurotoxins, and (ii) is an irreversible process upon which the capacity of AcChR membranes to bind α-Bgt cannot be regained. T_{50} values from heat inactivation curves seem to correlate well with DSC transition temperatures (T_{50}). Also, it is apparent that experimental conditions causing loss of cooperativity in the protein denaturation process can be detected either by DSC or by heat inactivation of the α-neurotoxin binding sites in similarly perturbed samples. Finally, the heat inactivation technique is sensitive to the presence of cholinergic agonists, antagonists, local anesthetics, and detergents in a qualitatively similar manner to that observed by DSC. This leads us to conclude that thermally induced loss of toxin binding sites on the AcChR protein is part of the main thermal event detected by DSC of AcChR membranes.

Thermal gel analysis of heat-inactivated samples provides information on thermally induced events at the level of AcChR subunits (31). The effects of heat on the observed aggregation of AcChR subunits suggests the occurrence of several thermally induced events in different regions of the AcChR protein exhibit little cooperativity with each other. It is also apparent that once the AcChR reaches a certain level of structural perturbation, there is a loss of the capacity to bind α-Bgt. The latter is a highly cooperative process, readily detected by DSC and sensitive to the presence of cholinergic agonists. These results are indicative of the complexity involved in the process of thermal denaturation of

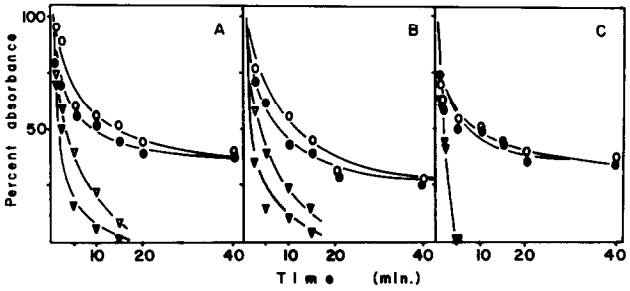

FIG. 9. Effect of heating at 60°C on the SDS–PAGE profiles exhibited by AcChR membranes which had been preincubated overnight, at 4°C, in the presence of 15 mM OG (A), 20 mM sodium cholate (B), or 10 mM sodium deoxycholate (C). Data are given as relative absorbances; 100% values correspond to the absorbance exhibited by each AcChR subunit as present in "control" unheated samples. Symbols for AcChR subunits are as in Fig. 8.

multimeric integral membrane proteins. The occurrence of similarly complex, thermally induced events has been reported for the membrane-bound cytochrome *c* oxidase (38).

We have used the combined heat inactivation–thermal gel procedure on AcChR samples to probe structural effects of subsolubilizing concentrations of detergents widely used for AcChR solubilization and reconstitution. All three detergents we have attempted produce a concentration-dependent thermal destabilization of the α-Bgt binding sites. According to current understanding of detergent action at low concentration (39), the observed effects could be caused through alteration of the membrane matrix resulting from incorporation of the detergent. This hypothesis is consistent with the observation that low concentration of one of the detergents used (OG), alters a specific membrane phenomenon: passive permeability to monovalent cations (Tl^+). Such a postulate, however, implies that modification of α-toxin binding sites, which are located 40–50 Å away from the membrane surface (40), would result from perturbation at transmembrane regions of AcChR. Similar conclusions were reached from studies using AcChR membranes in which a spectroscopic probe was covalently attached to the AcChR/lipid interface (41).

Detergent dialysis experiments indicate that structural AcChR alterations induced by detergents can be fully reversed when low concentrations of cholate or OG are present, but became apparently irreversible when deoxycholate is used as the perturbing agent. Our observations suggest that both cholate and OG are more appropriate than deoxycholate for AcChR solubilization and reconstitution experiments aimed at a minimal alteration of structural features of the AcChR protein.

The presence of detergents, but not local anesthetics, also increases the susceptibility to heat of AcChR subunit aggregation. Again, this is a concentration-dependent phenomenon, the extent of which is also dependent upon the type of detergent used. From the differences observed in the electrophoretic patterns caused by the presence of each detergent, it becomes apparent that deoxycholate is particularly effective in promoting a rapid heat-induced aggregation of the γ and δ subunits. The reasons for this are not understood at the moment but there is the possibility that damage to the more labile γ–δ pair, is related to the irreversible alteration observed in heat inactivation of α-toxin binding sites, when deoxycholate is used as the perturbing agent.

In conclusion, it seems that thermal perturbation techniques constitute a valid approach to probe specific structural alterations of the AcChR protein in its native membrane environment. Such information could be used in lipid dependence studies or in establishing structure/function relationships. Also, it is possible that similar heat inactivation techniques could be applied to study more heterogeneous membranes or reconstituted systems as long as specific ligands for the protein of interest are available.

ACKNOWLEDGMENTS

We thank Dr. M. Martinez-Carrion for kindly providing unlimited access to instrumentation at the Department of Biochemistry and Molecular Biophysics of the Medical College of Virginia. This work was partly supported by Grant 0726-84 from the "Comision Asesora para la Investigacion Cientifica y Technologica" of Spain.

REFERENCES

1. CONTI-TRONCONI, B. M., AND RAFTERY, M. A. (1982) *Annu. Rev. Biochem.* **51**, 491–530.
2. BARRANTES, F. J. (1983) *Int. Rev. Neurobiol.* **24**, 259–341.
3. HESS, G. P., CASH, D. J., AND AOSHIMA, H. (1983) *Annu. Rev. Biophys. Bioeng.* **12**, 443–473.
4. POPOT, J. L., AND CHANGEUX, J. P. (1984) *Physiol. Rev.* **64**, 1162–1239.
5. CHANG, H. W., AND BOCK, E. (1977) *Biochemistry* **16**, 4513–4518.
6. HAMILTON, S. L., MCLAUGHLIN, M., AND KARLIN, A. (1979) *Biochemistry* **18**, 155–159.
7. DALZIEL, A. W., ROLLINS, E. S., AND MCNAMEE, M. G. (1980) *FEBS Lett.* **122**, 193–197.
8. CRIADO, M., EIBL, H., AND BARRANTES, F. J. (1984) *J. Biol. Chem.* **259**, 9188–9198.
9. FONG, T. H., AND MCNAMEE, M. G. (1986) *Biochemistry* **25**, 830–840.

10. MOORE, H. P., AND RAFTERY, M. A. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4509–4514.
11. GONZALEZ-ROS, J. M., FERRAGUT, J. A., AND MARTINEZ-CARRION, M. (1984) *Biochem. Biophys. Res. Commun.* **120,** 368–373.
12. HESS, G. P., CASH, D. J., AND AOSHIMA, H. (1979) *Nature (London)* **282,** 329–331.
13. NEUBIG, R. R., AND COHEN, J. B. (1980) *Biochemistry* **19,** 2770–2779.
14. CASH, D. J., AND HESS, G. P. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 842–846.
15. NEUBIG, R. R., BOYD, N. D., AND COHEN, J. B. (1982) *Biochemistry* **21,** 3460–3467.
16. HESS, G. P., PASQUALE, E. B., WALKER, J. W., AND MCNAMEE, M. G. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 963–967.
17. HAMILL, O. P., AND SAKMANN, B. (1981) *Nature (London)* **294,** 462–464.
18. SACHS, F., AND AUERBACH, A. (1983) *in* Methods in Enzymology (Conn, M., Ed.), Vol. 103, Part H, Academic Press, New York.
19. CORONADO, R., AND LATORRE, R. (1983) *Biophys J.* **43,** 231–236.
20. MONTAL, M., LABARCA, P., FREDKIN, D. R., SUAREZ-ISLA, B. A., AND LINDSTROM, J. (1984) *Biophys. J.* **45,** 165–174.
21. MOORE, W. M., HOLLADAY, L. A., PUETT, D., AND BRADY, R. N. (1974) *FEBS Lett.* **45,** 145–149.
22. LIPERTT, J. L., LINDSAY, M., AND SCHULTZ, R. (1981) *J. Biol. Chem.* **256,** 12411–12416.
23. YAGER, P., CHANG, E. L., WILLIAMS, R. W., AND DALZIEL, A. W. (1984) *Biophys. J.* **45,** 26–28.
24. FARACH, M. C., AND MARTINEZ-CARRION, M. (1983) *J. Biol. Chem.* **258,** 4166–4170.
25. NEUBIG, R. R., KRODEL, E. K., BOYD, N. D., AND COHEN, J. B. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 690–694.
26. MARTINEZ-CARRION, M., GONZALEZ-ROS, J. M., MATTINGLY, J. R., FERRAGUT, J., FARACH, M. C., AND DONNELLY, D. (1984) *Biophys. J.* **45,** 141–145.
27. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., AND RANDALL, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
28. SCHMIDT, J., AND RAFTERY, M. A. (1978) *Anal. Biochem.* **52,** 349–354.
29. FERRAGUT, J. A., GONZALEZ-ROS, J. M., PETERSON, D. L., WEIR, D. L., FRANSON, R. C., AND MARTINEZ-CARRION, M. (1984) *Arch. Biochem. Biophys.* **235,** 628–635.
30. LYSKO, K. A., CARLSON, R., TAVERNA, R., SNOW, J., AND BRANDTS, J. F. (1981) *Biochemistry* **20,** 5570–5576.
31. SOLER, G., MATTINGLY, J. R., AND MARTINEZ-CARRION, M. (1984) *Biochemistry* **23,** 4630–4636.
32. LAEMMLI, N. K. (1970) *Nature (London)* **227,** 680–683.
33. DONNELLY, D., MIHOVILOVIC, M., GONZALEZ-ROS, J. M., FERRAGUT, J. A., RICHMAN, D., AND MARTINEZ-CARRION, M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7999–8003.
34. KARLIN, A., KAO, P. N., AND DIPAOLA, M. (1986) *TIPS* **7,** 304–308.
35. HELENIUS, A., AND SIMONS, K. (1975) *Biochim. Biophys. Acta* **415,** 29–79.
36. MIMMS, L. T., ZAMPIGHI, G., NOZAKI, Y., TANFORD, C., AND REYNOLDS, J. A. (1981) *Biochemistry* **20,** 833–840.
37. NODA, M., TAKAHASHI, H., TANABE, T., TOYOSATO, T., HIROSE, T., ASAI, M., TAKASHIMA, H., INAYAMA, S. MIYATA, T., AND NUMA S. (1983) *Nature (London)* **302,** 528–532.
38. RIGELL, C. W., SAUSSURE, C., AND FREIRE, E. (1985) *Biochemistry* **24,** 5638–5646.
39. HJELMELAND, L. M., AND CHRAMBACH, A. (1984) *in* Membranes, Detergents and Receptor Solubilization (Venter, J. C., and Harrison, L. C., Eds.), pp. 35–46, Alan R. Liss, New York.
40. KISTLER, J., STROUD, R. M., KLIMKOWSKY, M. W., LALANCETTE, R. A., AND FAIRCLOUGH, R. H. (1982) *Biophys. J.* **37,** 371–383.
41. GONZALEZ-ROS, J. M., FARACH, M. C., AND MARTINEZ-CARRION, M. (1983) *Biochemistry* **22,** 3807–3812.