measurements of chloride concentration and transients in cultured cells and in intact epithelia. One area in which this method may prove immediately useful is in cystic fibrosis research, where the suspected pathological lesion is a defect in the chloride conductance of tracheal epithelial cells (Welsh, 1986; Frizzell et al., 1986). A rapid and reliable assay for the chloride conductance that requires only a small quantity of tissue may be possible with SPQ.

ACKNOWLEDGMENTS

We thank Floyd C. Rector, Brian Davis, and Jay Nadel for their support and advice.

REFERENCES

Cabrini, G., Illsley, N. P., & Verkman, A. S. (1986) *Biochemistry 25*, 6300–6305.
Cupoletti, J., & Sachs, G. (1984) *J. Biol. Chem. 259*, 14952–14959.
DiStefano, A., Wittner, M., Schlatter, E., Lang, H. J., Englert, H., & Greger, R. (1985) *Pflugers Arch. 405 (Suppl. 1)*, S95–S100.
Frizzell, R. A., Rechkemmer, G., & Shoemaker, R. L. (1986) *Science (Washington, D.C.) 233*, 558–560.
Geck, P., & Heinz, E. (1986) *J. Membr. Biol. 91*, 97–105.
Illsley, N. P., & Verkman, A. S. (1986a) *Biophys. J. 49*, 156a.
Illsley, N. P., & Verkman, A. S. (1986b) *J. Membr. Biol. 94*, 267–278.
Illsley, N. P., Chen, P.-Y., & Verkman, A. S. (1987a) *Kidney Int. 31*, 437 (Abstr.).
Illsley, N. P., Lin, H. Y., & Verkman, A. S. (1987b) *Biochemistry 26*, 446–454.
Knauf, P. A. (1986) in *Physiology of Membrane Disorders* (Andreoli, T. E., Hoffman, J. H., Fanestil, D. D., & Schultz, S. G., Eds.) pp 191–220, Plenum Medical, New York.
Lakowicz, J. R. (1983) in *Principles of Fluorescence Spectroscopy*, pp 257–295, Plenum, New York.
Lakowicz, J. R., Cherek, H., & Balter, A. (1981) *J. Biochem. Biophys. Methods 5*, 131–146.
Ruzycki, S. M., Kelley, L. K., & Smith, C. H. (1978) *Am. J. Physiol. 234*, C27–C35.
Shennan, D. B., Davis, B., & Boyd, C. A. R. (1986) *Pflugers Arch. 406*, 60–64.
Steck, T. L., & Kant, J. A. (1974) *Methods Enzymol. 31*, 172–177.
Welsh, M. J. (1986) *Science (Washington, D.C.) 232*, 1648–1650.
Wolfbeis, O. S., & Urbano, E. (1982) *J. Heterocycl. Chem. 19*, 841–843.
Wolfbeis, O. S., & Urbano, E. (1983) *Fresenius' Z. Anal. Chem. 314*, 577–581.

Articles

Identification of Subunits of Acetylcholine Receptor That Interact with a Cholesterol Photoaffinity Probe†

David S. Middlemas‡ and Michael A. Raftery*
Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125
Received September 4, 1986; Revised Manuscript Received November 5, 1986

ABSTRACT: All four subunits of the acetylcholine receptor in membrane vesicles isolated from *Torpedo californica* have been labeled with [^{3}H]cholesteryl diazoacetate. As this probe incorporates into lipid bilayers analogously to cholesterol, this result indicates that acetylcholine receptor interacts with cholesterol. This investigation also demonstrates that this probe is a useful reagent for studying the interaction of cholesterol with membrane proteins.

The nicotinic acetylcholine receptor (AChR)[1] is an integral membrane protein that transduces a signal mediated by ACh into a membrane depolarization. This depolarization is the result of a transient current caused by the opening of an ion channel [for recent review, see Adams (1981)]. A unique opportunity to correlate the molecular structure of a gated ion channel with its functional properties is afforded by the availability of membrane vesicles greatly enriched in AChR from *Torpedo* electric organ [for recent reviews, see Conti-Tronconi and Raftery (1982) and Raftery et al. (1983)].

The AChR isolated from *Torpedo californica* is a pentameric complex of homologous subunits with apparent M_r of 40K, 50K, 60K, and 65K in a stoichiometry of 2:1:1:1 (Raftery et al., 1980). Several experimental approaches have shown that *T. californica* AChR is a transmembrane protein. Extracellular exposure of AChR was demonstrated by using anti-AChR antibodies conjugated with ferritin visualized by electron microscopy (Karlin et al., 1978). In other electron microscopic studies, antigenic determinants were revealed on both sides of the electroplax membrane (Strader et al., 1979; Tarrab-Hazdai et al., 1978). Exposure of the 40K, 50K, and 60K subunits to the aqueous phase was shown by labeling with

† This research was supported by grants from the National Institutes of Health (NS 10294) and the Department of the Army (DFAMD17-82-C-2175) and National Service Research Award 5 T32 GM07616-08 from the National Institute of General Medical Sciences.
‡ Present address: Molecular Biology and Virology Laboratory, The Salk Institute, San Diego, CA 92138.

[1] Abbreviations: AChR, acetylcholine receptor; ACh, acetylcholine; BrACh, bromoacetylcholine; MBTA, (4-*N*-maleimidobenzyl)trimethylammonium iodide; ESR, electron spin resonance; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; α-BgTx, α-bungarotoxin; THF, tetrahydrofuran; TEA, triethylamine; UV, ultraviolet; TLC, thin-layer chromatography; $NaDodSO_4$, sodium dodecyl sulfate; NMR, nuclear magnetic resonance; IR, infrared.

0006-2960/87/0426-1219$01.50/0

^{125}I using lactoperoxidase (Hartig & Raftery, 1977). Although trypsin added to the outside of *Torpedo marmorata* vesicles resulted in no degradation, after sonication, the three subunits present in the preparation were degraded, suggesting their intracellular exposure (Wennogle & Changeux, 1980). Definitive evidence for the transmembrane nature of all four subunits of *T. californica* AChR was obtained by proteolysis with trypsin (Strader & Raftery, 1980). Trypsin added both externally and internally to sealed vesicles, 95% of which had their synaptic surface facing the external medium, resulted in proteolysis of all four subunits. Although both external exposure to trypsin and internal exposure to trypsin cause significant subunit degradation, neither alters agonist-induced flux properties (Conti-Tronconi et al., 1982). This lends credence to the conclusion that proteolysis occurs at tryptic cleavage sites exposed on both sides of the membrane rather than being the result of an artifactual exposure of cleavage sites caused by degradation and subsequent perturbation of the AChR–lipid complex.

The exposure of AChR to the lipid bilayer has been studied by using photoactivated probes that partition predominantly into the lipid bilayer. 5-[^{125}I]Iodonaphthyl 1-azide labeled exclusively the 40K subunit (Tarrab-Hazdai et al., 1980) while [^{3}H]pyrenesulfonyl azide labeled polypeptides of 48K and 55K (Šator et al., 1979; Gonzalez-Ros et al., 1979). This difference in labeling pattern may be due to different reactivity of nitrenes. By use of [^{3}H]adamantanediazirine, all four subunits of the AChR were labeled, demonstrating exposure of all four subunits to the hydrocarbon core of the lipid bilayer (Middlemas & Raftery, 1983). [^{3}H]Adamantanediazirine, which generates a carbene upon photolysis, is probably a more effective hydrophobic probe than the alternative probes, which generate nitrenes (Bayley & Knowles, 1980). This result agrees with the sequence data of Noda et al. (1982, 1983a,b), which show that all four subunits contain putative membrane-spanning regions.

It is probable that AChR is a pseudosymmetric complex of homologous subunits, all of which interact with the lipid bilayer in a similar manner. The role of lipids in the function and structure of the AChR remains an important area of research. There is evidence that cholesterol is important to AChR function. In reconstitution experiments, inclusion of cholesterol was needed for protection of agonist-induced change in affinity of binding sites (desensitization) (Criado et al., 1982) and protection of agonist-induced $^{86}Rb^{+}$ flux (Dalziel et al., 1980). By use of a spin-labeled steroid probe, an immobilized component was revealed by ESR spectra, which suggests AChR interacts with neutral lipids (Marsh & Barrantes, 1978).

Although cholesterol may be important to AChR function, it is not clear whether this is due to its effect on membrane properties or a specific interaction with AChR. In this paper, we have used the cholesterol photoaffinity probe, cholesteryl diazoacetate, to resolve whether or not cholesterol interacts with AChR. Cholesteryl diazoacetate incorporates into lipid bilayers analogously to cholesterol and is therefore a direct tool to study the interaction of cholesterol with both lipids and membrane proteins. It is immobilized in bilayers like cholesterol and upon irradiation incorporates into the choline head group of phosphatidylcholine (Keilbaugh & Thornton, 1983a,b). This result supports the notion that the carbene is generated near the lipid–water interface.

Materials and Methods

Membrane fragments enriched in AChR were prepared from electric organs from *T. californica* as described by Elliott et al. (1980) and were further purified by alkali extraction (Neubig et al., 1979; Elliott et al., 1979). The membranes were finally suspended in Ca^{2+}-free *Torpedo* Ringer's solution adjusted to pH 7.4 (250 mM NaCl, 5 mM KCl, 20 mM HEPES, 0.02% NaN_3, and 2 mM $MgCl_2$). Protein concentration was determined by the method of Lowry et al. (1951). The concentration of α-BgTx sites was determined by the method of Schmidt and Raftery (1973) using ^{125}I-α-BgTx obtained from New England Nuclear and calibrated by the procedures of Blanchard et al. (1979). The specific activities of the preparations varied between 2.0 and 3.7 nmol of α-BgTx binding sites (mg of protein)$^{-1}$. All tritium samples were counted on a Beckman LS233 counter following addition of 10 mL of Aquasol 2 (New England Nuclear).

Synthesis and Purification of [^{3}H]Cholesteryl Diazoacetate. Cholesteryl diazoacetate was synthesized and purified by the method of Keilbough and Thornton (1983a). A similar method was used to synthesize [^{3}H]cholesteryl diazoacetate from [7-^{3}H]cholesterol (12 Ci mmol^{-1}) purchased from ICN Pharmaceuticals, Inc. A sample of 2.8 μmol of cholesterol was added to 5.3 mCi of [7-^{3}H]cholesterol in benzene (1 mCi mL^{-1}). The solvent was removed under reduced pressure at 22 °C. The cholesterol was dissolved in 14 μL of CH_2Cl_2/THF (1:1) containing 5 μmol of redistilled TEA on ice. A sample of 5.2 μmol of glyoxylic acid chloride *p*-toluenesulfonyl hydrazone, prepared by the method of Blankley et al. (1969), was added in 17 μL of CH_2Cl_2 cooled to 0 °C. After 30 min in the dark on ice, 5 μmol of TEA in 5 μL of CH_2Cl_2 was added. The solution was brought to 22 °C. After 30 min, the solvent was removed under reduced pressure at 22 °C. The residue was extracted twice with 100 μL of toluene. The extracts were applied to a 0.5 × 4 cm silica gel 60 column (Merck) and eluted with toluene. Toluene was removed from the purified diazoacetate under reduced pressure. The diazoacetate was taken up in ethanol for determination of the specific activity and introduction into membranes.

The specific activity of [^{3}H]cholesteryl diazoacetate was determined by using UV absorbance at 247 nm (ϵ_{247} = 17 000) and scintillation counting with $^{3}H_2O$ as an internal standard. Plastic-backed silica gel 60 TLC plates (Merck), which were eluted with toluene and then sliced and counted, were used to verify the purity of the tritiated diazoacetate. The unlabeled diazoacetate was visualized on TLC plates with I_2.

Photolysis of Cholesteryl Diazoacetate. Photolysis was carried out in a quartz cuvette with a UVSL-25 lamp (Ultraviolet Products) on the short-wavelength setting. The rate of photolysis of cholesteryl diazoacetate in ethanol and cyclohexane was monitored by the absorbance at 247 nm. The rate of photolysis of the tritiated diazoacetate incorporated into vesicles enriched in AChR was monitored by taking time aliquots for TLC. When eluted with toluene, the diazoacetate migrated near the solvent front while the products of photolysis remained near the origin. The TLC plates were then sliced and counted.

Labeling of Membrane Vesicles. All photolabeling experiments were carried out in Ca^{2+}-free *Torpedo* Ringer's solution with constant stirring at room temperature. [^{3}H]Cholesteryl diazoacetate in ethanol was added to membrane vesicles in a quartz cuvette under dim light. The final concentrations of [^{3}H]Cholesteryl diazoacetate, membrane protein, and ethanol were 12 μM, 1 mg mL^{-1}, and 1%, respectively. The sample was incubated in the dark with stirring for 30 min, and then the cuvette was flushed with argon. The sample was irradiated for 10 min by using a UVSL-25 lamp on the short-wavelength setting with constant stirring. The membranes were then

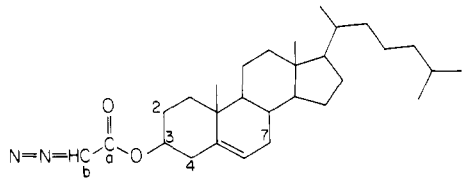

FIGURE 1: Structure of cholesteryl diazoacetate.

pelleted by centrifuging for 15 min in an Eppendorf centrifuge, after which the samples were taken up in buffer for gel electrophoresis.

Membrane vesicles desensitized with 10 μM carbamylcholine and 4 mM $CaCl_2$ were photolabeled as described. Desensitization was shown by the associated inhibition of the time course of α-BgTx–receptor complex formation by using the procedure of Lee et al. (1977).

Extraction of lipids from the protein was accomplished by adding 20 volumes of chloroform/methanol (2:1) to 1 volume of photolyzed membranes in a glass centrifugation tube. After vortexing, the samples were centrifuged at 12000*g* for 30 min. The supernatant was removed, and the pellet was dissolved in buffer for gel electrophoresis. Aliquots of both the supernatant and pellet were taken for scintillation counting. Quenching was accounted for by using ^3H_2O as an internal standard.

Gel Electrophoresis. $NaDodSO_4$–polyacrylamide gel electrophoresis was performed according to the system of Laemmli (1970) with 10% acrylamide cylindrical gels (0.5 × 10 cm). After electrophoresis, gels were immediately sliced with a Hoeffer Scientific Instruments gel slicer. Each slice was sealed in a glass scintillation vial with 0.5 mL of 30% H_2O_2 and incubated at 70 °C for 5 h, followed by determination of radioactivity. Electrophoresis of unlabeled AChR preparations was performed simultaneously to determine the mobility of the subunits. These gels were stained with 0.5% Coomassie Blue in 10% acetic acid/25% propanol/65% H_2O and destained in the same solvent without the dye.

RESULTS

Cholesteryl diazoacetate was synthesized and purified by the method of Keilbaugh and Thornton (1983a) (Figure 1). IR and NMR spectra and melting point agreed with the published values. By use of a similar approach, [^{3}H]cholesteryl diazoacetate with a specific activity of 1.64 Ci mmol^{-1} was synthesized and purified (11% yield). The mobility on TLC, the UV spectrum, and the rate of photolysis of the tritiated compound were identical with those of cholesteryl diazoacetate. In both labeled and unlabeled cholesteryl diazoacetate, greater than 99% of the UV absorbance at 247 nm disappears upon photolysis.

In order to ensure complete photolysis, the rate of photolysis was determined under various conditions (Figure 2). The half-lives of photolysis in ethanol and cyclohexane are 47 and 76 s, respectively, and in membrane vesicles containing 1 mg mL^{-1} protein, the half-life of photolysis was 49 s. It is interesting to note that the rate of photolysis is similar both in organic solvent and in unilamellar vesicles, although much longer irradiation times are required for photolysis in multilamellar vesicles (Keilbaugh & Thornton, 1983b). It is evident that exposure to UV light under these conditions will result in virtually complete photolysis of the diazo group in 10 min.

After incorporation of [^{3}H]cholesteryl diazoacetate into AChR-enriched membrane vesicles, irradiation resulted in covalent labeling of all four subunits (Figure 3). The ratio of label migrating at the dye front, which corresponds to both labeled lipids and cholesterol, to label incorporated into protein

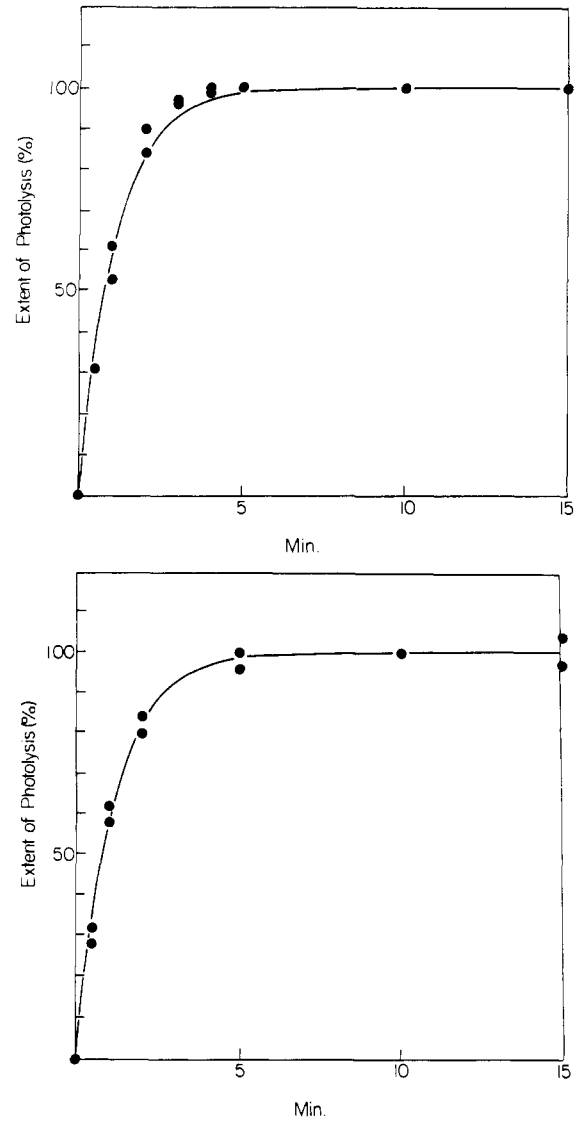

FIGURE 2: Time course of photolysis of cholesteryl diazoacetate in ethanol (top) and [^{3}H]cholesteryl diazoacetate in membrane vesicles (bottom).

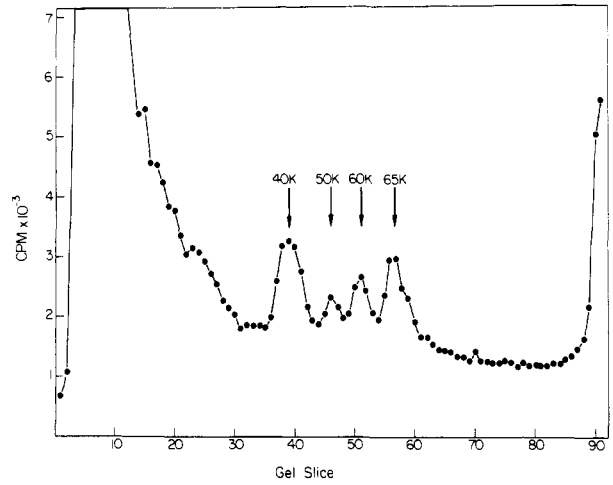

FIGURE 3: $NaDodSO_4$–polyacrylamide gel electrophoresis scan of AChR labeled with [^{3}H]cholesteryl diazoacetate in membrane vesicles.

is 75. Since much of the carbene will react with water to form cholesterol esters and cholesterol, the ratio above does not indicate the ratio of carbene insertion into lipids over proteins. The high background radioactivity is presumably caused by tritiated cholesterol esters and cholesterol (which migrate with

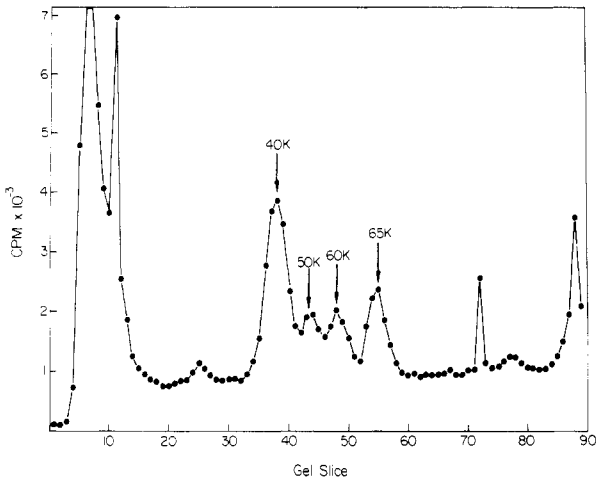

FIGURE 4: $NaDodSO_4$–polyacrylamide gel electrophoresis scan of AChR labeled with [^{3}H]cholesteryl diazoacetate in membrane vesicles after extraction of lipids with chloroform/methanol.

the dye front presumably in detergent micelles), and therefore, a chloroform/methanol extraction of both labeled lipids and cholesterol from the protein prior to electrophoresis was used to reduce this background. Although the radioactive lipids were not quantitatively extracted (Figure 4), about 70% of the radioactivity was extracted with the chloroform/methanol. The stoichiometry of covalent incorporation into the 40K, 50K, 60K, and 65K subunits, respectively, was about 4:1:1:2, i.e., a molar ratio of 2:1:1:2 (Figure 4). As all four subunits should be precipitated during the extraction in a quantitative manner, this ratio is likely to be correct.

Since there is evidence that cholesterol may be important in desensitization or at least essential for protection of desensitization properties of receptor during reconstitution experiments, the effect of agonist on the interaction of [^{3}H]-cholesteryl diazoacetate with AChR was investigated. The probe was incorporated into AChR-enriched vesicles, which were desensitized by exposure to 10 μM carbamylcholine as verified by inhibition of ^{125}I-α-BgTx binding. Irradiation of both desensitized and control AChR-enriched membrane vesicles resulted in an identical distribution of label among the four subunits.

In the above experiments, [^{3}H]cholesteryl diazoacetate was introduced into membranes in small portions of ethanol. An alternative method of introducing the probe was used to investigate the possibility of the label distribution being affected by the ethanol. The probe was introduced into the cuvette, and ethanol was removed with a gentle stream of nitrogen. Membrane suspension was then introduced and stirred at 22 °C for 4 h. Although complete uptake of the probe from the walls of the cuvette was not possible, all four subunits were labeled after irradiation. This indicates that ethanol does not affect the labeling of AChR with [^{3}H]cholesteryl diazoacetate.

Discussion

ESR experiments with a labeled steroid probe indicated an interaction of neutral lipids with AChR (Marsh & Barrantes, 1978). By use of an androstanol spin-labeled probe, the electron spin resonance spectra revealed an immobilized component along with the fluid component that is found in aqueous dispersions of extracted lipids. A similar result was obtained with stearic acid probes. AChR has been shown to incorporate more readily into cholesterol monolayers than other lipid monolayers, suggesting that it has an affinity for cholesterol (Popot et al., 1978). The photolabeling results presented in this paper from using [^{3}H]cholesteryl diazoacetate demonstrate definitively that cholesterol interacts with AChR.

Investigation of the distribution of cholesterol in membranes is possible by using saponin, filipin and digitonin, which form complexes with cholesterol that can be identified by electron microscopy after freeze–fracture (Elias et al., 1978, 1979). Several groups have found little or no evidence for the presence of cholesterol in AChR-rich regions of membranes, using these probes. However, in one study, evidence for the presence of cholesterol in areas of membrane rich in AChR clusters was found. In both noninnervated and innervated *Xenopus* embryonic muscle cells, there was virtually no indication of cholesterol complexes in areas rich in aggregates of AChR particles (Bridgman & Nakajima, 1981). In *Rana* cutaneous pectoris and sartorius muscles, filipin–sterol complexes were absent from regions occupied with AChR aggregates (Nakajima & Bridgman, 1981), and there was also an almost complete absence of filipin–sterol complexes in AChR-rich regions of the electroplax membrane of *T. marmorata* (Perrelet et al., 1982). However, in rat myotubes, areas of membrane rich in AChR clusters reacted extensively with filipin, which indicates the presence of cholesterol (Pumplin & Bloch, 1983), although in the same study, membrane rich in AChR clusters in chick myotubes did not react with saponin or filipin (Pumplin & Bloch, 1983).

The role that cholesterol plays in AChR function has been investigated. Inclusion of cholesterol during reconstitution was found to be essential to protect agonist-induced affinity change of cholinergic binding sites (Criado et al., 1982). In another study it was reported that cholesterol was necessary for the maintenance of agonist-induced $^{86}Rb^+$ flux (Dalziel et al., 1980). Zabrecky and Raftery (1985) found the binding affinity of AChR for carbamylcholine increased as the cholesterol content of membrane vesicles was reduced. The cholesterol content was reduced by fusion of AChR-enriched vesicles with phospholipid vesicles of defined composition. Although it is apparent that cholesterol is important to AChR function, it is not clear whether this is because of its effect on membrane properties or a specific interaction with AChR.

A recent study, using photoreactive phospholipid probes, found that all four subunits of both *T. californica* and *T. marmorata* AChR were labeled (Giraudat et al., 1985). The study clearly demonstrated that all four subunits interact with charged lipids. Our results, which indicate neutral lipids interact with the AChR, are consistent with these findings.

Cholesteryl diazoacetate is a useful reagent for the study of membrane proteins. It is readily photolyzed and exhibits a low level of Wolff rearrangement (Keilbaugh & Thornton, 1983a). Reasonable yields of incorporation into lipids and membrane proteins can be obtained. Since the photogenerated carbene is situated near the lipid–water interface (Keilbaugh & Thornton, 1983b), this probe has potential as a topographic tool to map membrane protein structure. By use of a photoactivated lipid probe, which also generates a carbene near the lipid–water interface, regions of glycophorin A near the interface have been determined (Ross et al., 1982). As cholesteryl diazoacetate incorporates into membrane proteins and lipids, it is useful in the study of the interaction of cholesterol with both lipids and membrane proteins.

In this paper, we have demonstrated covalent labeling of AChR with [^{3}H]cholesteryl diazoacetate. Since this probe incorporates into lipid bilayers in a fashion analogous to that of cholesterol, this result confirms that AChR interacts with cholesterol. It is interesting to note that the labeling of AChR with this probe is very similar to the labeling with [^{3}H]-adamantanediazirine, a probe that labels proteins exposed to

the hydrocarbon core of the lipid bilayer (Middlemas & Raftery, 1983). As both of these reagents label all of the subunits, it is probable that all of the subunits interact with the membrane in a related manner.

ACKNOWLEDGMENTS

We thank J. Racs for valuable technical assistance.

Registry No. *p*-$MeC_6H_4SO_2NHN{=}CHCOCl$, 14661-69-9; [^{3}H]cholesteryl diazoacetate, 106471-96-9; cholesterol, 57-88-5; [7-^{3}H]cholesterol, 82730-32-3.

REFERENCES

Adams, P. R. (1981) *J. Membr. Biol. 58*, 161–174.

Bayley, H., & Knowles, J. R. (1980) *Biochemistry 19*, 3883–3892.

Blanchard, S. G., Quast, U., Reed, K., Lee, T., Schimerlik, M. I., Vandlen, R., Claudio, T., Strader, C. D., Moore, H.-P. H., & Raftery, M. A. (1979) *Biochemistry 18*, 1875–1883.

Blankley, C. J., Sauter, F. J., & House, H. O. (1969) *Org. Synth. 49*, 22–27.

Bridgman, P. C., & Nakajima, Y. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 1278–1282.

Conti-Tronconi, B. M., & Raftery, M. A. (1982) *Annu. Rev. Biochem. 51*, 491–530.

Conti-Tronconi, B. M., Dunn, S. M. J., & Raftery, M. A. (1982) *Biochemistry 21*, 893–899.

Criado, M., Eibl, H., & Barrantes, F. J. (1982) *Biochemistry 21*, 3622–3629.

Dalziel, A. W., Rollins, E. S., & McNamee, M. G. (1980) *FEBS Lett. 122*, 193–196.

Elias, P. M., Goerke, J., Friend, D. S., & Brown, B. E. (1978) *J. Cell Biol. 78*, 577–596.

Elias, P. M., Friend, D. S., & Goerke, J. (1979) *J. Histochem. Cytochem. 27*, 1247–1260.

Elliott, J., Dunn, S. M. J., Blanchard, S. G., & Raftery, M. A. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 2576–2579.

Elliott, J., Blanchard, S. G., Wu, W., Miller, J., Strader, C. D., Hartig, P., Moore, H.-P., Racs, J., & Raftery, M. A. (1980) *Biochem. J. 185*, 667–677.

Giraudat, J., Montecucco, C., Bisson, R., & Changeux, J.-P. (1985) *Biochemistry 24*, 3121–3127.

Gonzalez-Ros, J. M., Calvo-Fernandez, P., Šator, V., & Martinez-Carrion, M. (1979) *J. Supramol. Struct. 11*, 327–338.

Hartig, P. R., & Raftery, M. A. (1977) *Biochem. Biophys. Res. Commun. 78*, 16–22.

Karlin, A., Holtzman, E., Valderrama, R., Damle, V., Hsu, K., & Reyes, F. (1978) *J. Cell Biol. 76*, 577–592.

Keilbaugh, S. A., & Thornton, E. R. (1983a) *J. Am. Chem. Soc. 105*, 3283–3286.

Keilbaugh, S. A., & Thornton, E. R. (1983b) *Biochemistry 22*, 5063–5068.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Lee, T., Witzeman, V., Schimerlik, M. I., & Raftery, M. A. (1977) *Arch. Biochem. Biophys. 183*, 57–63.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Marsh, D., & Barrantes, F. J. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4329–4333.

Middlemas, D. S., & Raftery, M. A. (1983) *Biochem. Biophys. Res. Commun. 115*, 1075–1082.

Nakajima, Y., & Bridgman, P. C. (1981) *J. Cell Biol. 88*, 453–458.

Neubig, R. R., Krodel, E. K., Boyd, N. D., & Cohen, J. B. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 690–694.

Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Furutani, Y., Hirose, T., Asai, M., Inayama, S., Miyata, T., & Numa, S. (1982) *Nature (London) 299*, 793–797.

Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Kikyotani, S., Hirose, T., Asai, M., Takashima, H., Inayama, S., Miyata, T., & Numa, S. (1983a) *Nature (London) 301*, 251–255.

Noda, M. Takahashi, H., Tanabe T., Toyosato, M., Kikyotani, S., Furutani, Y., Hirose, T., Takashima, H., Inayama, S., Miyata, T., & Numa, S. (1983b) *Nature (London) 302*, 528–532.

Perrelet, A., Garcia-Segura, L.-M., Singh, A., & Orci, L. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 2598–2602.

Popot, J.-L., Demel, R. A., Sobel, A., Van Deenen, L. L. M., & Changeux, J.-P. (1978) *Eur. J. Biochem. 85*, 27–42.

Pumplin, D. W., & Bloch, R. J. (1983) *J. Cell Biol. 97*, 1043–1054.

Raftery, M. A., Hunkapiller, M. W., Stader, C. D., & Hood, L. E. (1980) *Science (Washington, D.C.) 208*, 1454–1457.

Raftery, M. A., Dunn, S. M. J., Conti-Tronconi, B. M., Middlemas, D. S., & Crawford, R. D. (1983) *Cold Spring Harbor Symp. Quant. Biol. 48*, 21–33.

Ross, A. H., Radhakrishnan, R., Robson, R. J., & Khorana, H. G. (1982) *J. Biol. Chem. 257*, 4152–4161.

Šator, V., Gonzalez-Ros, J. M., Calvo-Fernandez, P., & Martinez-Carrion, M. (1979) *Biochemistry 18*, 1200–1206.

Schmidt, J., & Raftery, M. A. (1973) *Anal. Biochem. 52*, 349–354.

Strader, C. D., & Raftery, M. A. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5807–5811.

Strader, C. D., Revel, J.-P., & Raftery, M. A. (1979) *J. Cell Biol. 83*, 499–510.

Tarrab-Hazdai, R., Geiger, B., Fuchs, S., & Amsterdam, A. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 2497–2501.

Tarrab-Hazdai, R., Bercovici, T., Goldfarb, V., & Gitler, C. (1980) *J. Biol. Chem. 255*, 1204–1209.

Wennogle, L. P., & Changeux, J.-P. (1980) *Eur. J. Biochem. 106*, 381–393.

Zabrecky, J. R., & Raftery, M. A. (1985) *J. Recept. Res. 5*, 397–417.