Gascoigne, I. M., & Radda, G. K. (1967) *Biochim. Biophys. Acta 131*, 498-507.
Hamilton, G. A. (1971) *Prog. Bioorg. Chem. 3*, 83-157.
Hemmerich, P. (1976) *Fortschr. Chem. Org. Naturst. 33*, 451-527.
Lee, C., Eichner, R. D., & Kaplan, N. O. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 1593-1597.
Matthews, R. G., Wilkinson, K. D., Ballou, D. P., & Williams, Ch. H., Jr. (1976) in *Flavins and Flavoproteins, Proceedings of the International Symposium, 5th, 1975* (Singer, T. P., Ed.) pp 464-472, Elsevier Scientific Publishing Co., New York.
Matthews, R. G., Ballou, D. P., & Williams, C. H., Jr. (1979) *J. Biol. Chem. 254*, 4974-4981.
Muller, F., & Massey, V. (1969) *J. Biol. Chem. 244*, 4007-4016.
Redfield, A. G., & Gupta, R. K. (1971) *Cold Spring Harbor Symp. Quant. Biol. 36*, 405-411.
Thorpe, C., & Williams, C. H., Jr. (1976a) *J. Biol. Chem. 251*, 3553-3557.
Thorpe, C., & Williams, C. H., Jr. (1976b) *J. Biol. Chem. 251*, 7726-7728.
Thorpe, C., & Williams, C. H., Jr. (1981) *Biochemistry 20*, 1507-1513.
Williams, C. H., Jr. (1976) *Enzymes, 3rd Ed. 13*, 89-173.
Williams, C. H., Jr., Zanetti, G., Arscott, L. D., & McAllister, J. K. (1967) *J. Biol. Chem. 242*, 5226-5231.

Purification of Acetylcholine Receptors from the Muscle of *Electrophorus electricus*†

J. M. Lindstrom,* J. F. Cooper, and L. W. Swanson

ABSTRACT: Muscle from the electric eel *Electrophorus electricus* contains acetylcholine receptors at 50 times the concentration of normal mammalian muscle and fully one-tenth the concentration of receptors in its electric organ tissue. Receptor is organized much more diffusely over the surface of *Electrophorus* muscle cells than is the case in normally innervated mammalian skeletal muscle. Receptor was purified from *Electrophorus* muscle by affinity chromatography on cobra toxin-agarose and found to contain subunits which correspond immunochemically to the α, β, γ, and δ subunits of receptor from electric organ tissue of *Torpedo californica*. Receptor purified from *Electrophorus* muscle appears virtually identical with receptor purified from *Electrophorus* electric organ tissue.

Acetylcholine receptors were first purified from the electric organs of the electric eel *Electrophorus electricus* 10 years ago (Biesecker, 1973; Karlin & Cowburn, 1973; Klett et al., 1973; Meunier et al., 1974; Chang, 1974; Lindstrom & Patrick, 1974), but we only recently checked skeletal muscle of *Electrophorus* for its content of receptor. Surprisingly, it contains much more receptor than does innervated mammalian muscle, and even more than denervated or fetal muscle.

Previously we (Lindstrom et al., 1980) found that acetylcholine receptors purified from electric organ tissues of *Electrophorus* were composed of four subunits corresponding to the α, β, γ, and δ subunits of receptor from the electric organ tissue of the electric ray *Torpedo californica* (Weil et al., 1974; Raftery et al., 1975). Subsequent determination of the N-terminal amino acid sequence of each of these subunits showed that they were present in the same $\alpha_2\beta\gamma\delta$ stoichiometry (Reynolds & Karlin, 1978; Lindstrom et al., 1979a,b; Raftery et al., 1980) observed in receptor from *Torpedo* and that, as in *Torpedo*, there was substantial amino acid sequence homology between the α, β, γ, and δ subunits of *Electrophorus* (Conti-Tronconi et al., 1982). These results indicated that these subunits evolved by duplication and reduplication of a primordial gene and that the subunit stoichiometry was established before the divergence of primitive vertebrates into cartilaginous and bony fish some 400 million years ago.

Electric organ tissue evolved from muscle tissues independently in several genuses (Bennett, 1970; Mellinger et al., 1978). The presence of a relatively high concentration of receptor in *Electrophorus* gave us a chance to purify significant amounts of receptor from two related tissues in one animal and compare their structures. This is an especially interesting question because acetylcholine receptors in muscle are known to change several of their properties asynchronously during development (Dennis et al., 1981) and because receptor from normally innervated muscle differs in properties like channel open time (Sakman, 1978) or reaction with some antibodies (Weinberg & Hall, 1979) from receptors in denervated or fetal tissue, yet has apparently similar subunit structure (Nathanson & Hall, 1979; Sumikawa et al., 1982). These studies of receptor from normal and denervated muscle were complicated by the fact that only tiny amounts of receptor were obtained, and this was so proteolytically degraded that all four types of receptor subunits could not be detected. It has only recently become possible to purify significant amounts of receptor from fetal bovine muscle with α, β, γ, and δ subunits immunochemically demonstrable (Einarson et al., 1982). Muscle from *Electrophorus* has 4 times the receptor concentration of fetal bovine muscle. In addition to their relevance to studies of changes in receptor during muscle development, comparative studies of receptor from electric organ and muscle are relevant to studies of changes in receptor during evolution and provide

† From the Salk Institute for Biological Studies, San Diego, California 92138. *Received December 30, 1982.* This work was supported by grants from the National Institutes of Health (NS11323 and NS116686), The Muscular Dystrophy Association, The McKnight Foundation, the Los Angeles and California Chapters of the Myasthenia Gravis Foundation, and the Office of Naval Research. L.W.S. is a Clayton Foundation investigator.

0006-2960/83/0422-3796$01.50/0 © 1983 American Chemical Society