The Life History of Acetylcholine Receptors

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INTRODUCTION

The acetylcholine (ACh) receptor is the major integral membrane glycoprotein known to occur in the postsynaptic membrane at the neuromuscular junction. ACh receptors also occur in extrajunctional regions of embryonic and denervated muscle fibers (Fig. 1). Thus, the study of ACh receptor metabolism yields information pertinent to various scientific problems, including the mechanisms of membrane biogenesis, myogenesis, formation of synapses, and regulation of spatial distribution of cell surface functions. These matters have been reviewed in detail recently (Fambrough, 1979). Here we will describe briefly the life-history, as best we know it, of extrajunctional ACh receptor molecules. Presumably, the events in the life of junctional ACh receptors include most of the same events (plus some others), although the timing and duration of events may be different.

SITE OF BIOSYNTHESIS AND PROCESSING

Newly synthesized ACh receptors are first detected in the Golgi apparatus (Fig. 2) (Fambrough and Devreotes, 1978). At this stage the receptor units are already large glycoprotein molecules intimately associated with lipid bilayer (Devreotes and Fambrough, 1975; Patrick et al., 1977). Detergent solubilized receptor molecules at this stage resemble cell surface ACh receptors in size, affinity for ACh and (+)-tubocurarine and precipitability with concanavalin A and with antireceptor antisera.

Receptor molecules probably appear in the Golgi apparatus within about 15 min of the time of biosynthesis of their constituent polypeptide chains (Fambrough and Devreotes, 1978). Biosynthesis and assembly of receptor subunits to form complete receptors involves both protein synthesis and glycosylation. When the lipid intermediate pathway of protein glycosylation is blocked

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Fig. 1. Light-microscope autoradiograms showing the approximate location of cholinergic receptors in embryonic, adult and denervated adult muscles, as revealed by ^{125}I-labeled α-bungarotoxin binding. (a) Embryonic chick skeletal muscle in tissue culture. (b) Portion of single muscle fibre from 19-day embryonic chick anterior latissimus dorsi muscle, illustrating extrajunctional receptors and clusters of receptors at the periodic sites of multiple innervation. (C) End-plate region of single muscle fibre from normal adult human deltoid muscle. (d) Portion of muscle fibre from 10-day denervated rat diaphragm muscle, illustrating the continued presence of dense receptor clustering at former postsynaptic site (arrow) and also nearby extrajunctional receptor sites. Magnification bars represent 20 μm in (a), (b), and (d), 50 μm in (c). (From Fambrough et al., 1977).

by tunicamycin, receptor formation ceases, but receptors already in the Golgi apparatus continue their transport to the cell surface (Fambrough, 1977). As yet it is unknown whether biosynthesis of receptor subunits involves rough endoplasmic reticulum, which is a very minor organelle in muscle.

The intracellular pool of newly synthesized receptors contains those receptors which will appear in the plasma membrane during the next three hours

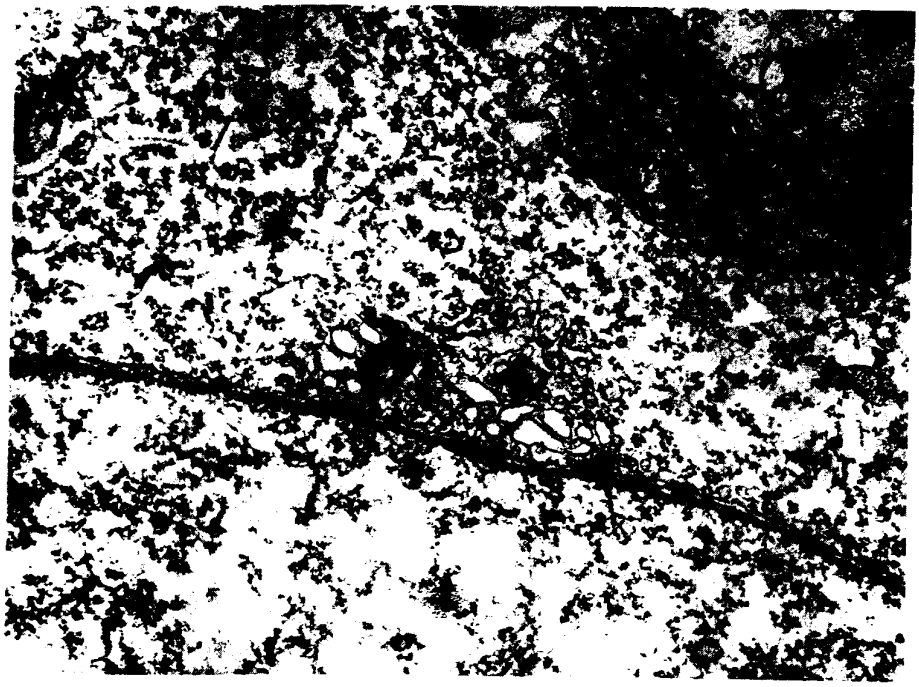

Fig. 2. Electron microscope autoradiograph illustrating the association of ACh receptors with the Golgi apparatus, as revealed by the binding of iodinated α-bungarotoxin to fixed, saponin-treated chick skeletal muscle cells in tissue culture.

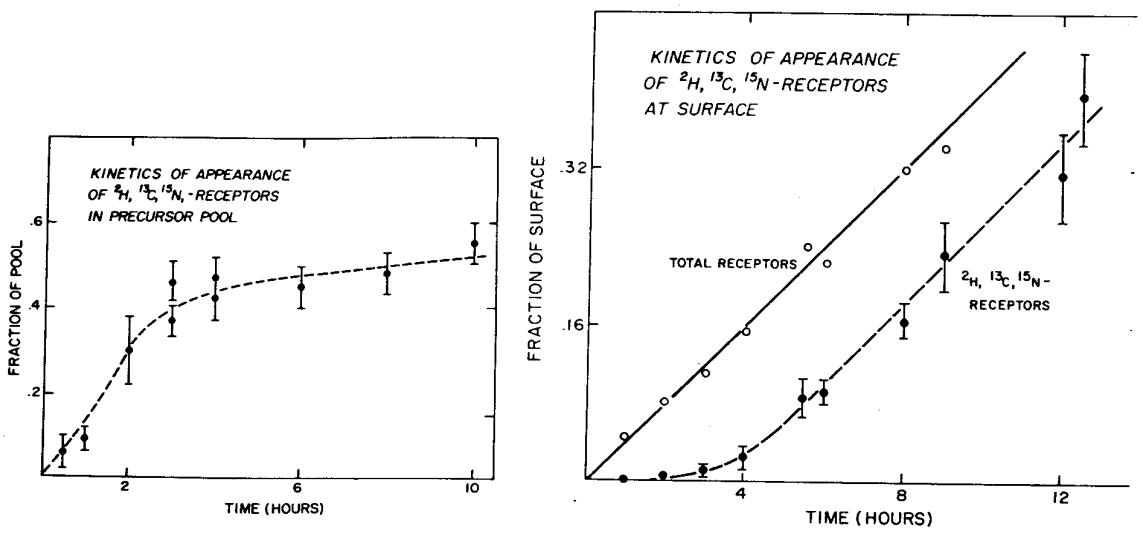

Fig. 3. (a) Kinetics of appearance of ^{2}H-, ^{13}C-, ^{15}N-receptors in the intracellular pool during incubation in medium containing ^{2}H-, ^{13}C-, ^{15}N-amino acids. (b) Kinetics of appearance of total (^{1}H-, ^{12}C-, ^{14}N-, plus ^{2}H-, ^{13}C-, ^{15}N-) and density-shifted (^{2}H-, ^{13}C-, ^{15}N-) receptors on myotube surfaces during incubation in medium containing ^{2}H-, ^{13}C-, ^{15}N-amino acids. (From Devreotes et al, 1977).

(Fig. 3) (Devreotes et al., 1977). In young myotubes in vitro, this pool contains 10 to 15% as many receptors as are present on the plasma membrane. Transport of ACh receptors to the plasma membrane possibly makes some nonessential use of microtubular elements: 3 μM colchicine slows the rate of receptor appearance in the plasma membrane about 50% while lumicolchicine has no effect and higher concentrations of colchicine are no more inhibitory (Rotundo and Fambrough, unpublished observations).

INCORPORATION INTO PLASMA MEMBRANE

Receptor molecules appear in the plasma membrane about 3 h after biosynthesis (Devreotes et al., 1977). This appearance probably involves fusion of membrane vesicles containing ACh receptor molecules with the plasma membrane. Before this event, receptor molecules are located in membrane-bound structures with the receptor sites protected from interaction with ligands (presumably because the receptor sites occur on the inner surface of closed vesicles) (Devreotes and Gardner, unpublished observations).

Incorporation of new receptors into the plasma membrane is an energy-requiring process as judged by rapid inhibition by compounds which interfere with ATP production. Low temperature also prevents incorporation (Fig. 4). In young myotubes the sites of incorporation are well distributed over the cell surface (Hartzell and Fambrough, 1973). In older myotubes in vitro there may be preferential incorporation at sites of ACh receptor clustering (Fischbach et al., 1976).

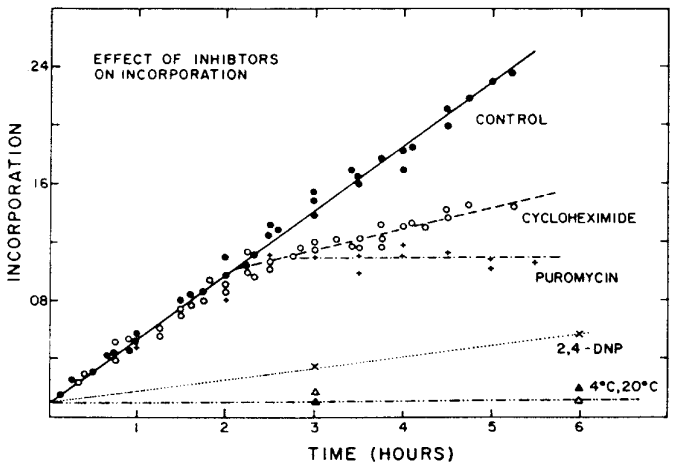

Fig. 4. Incorporation of new ACh receptors into plasma membranes of cultured chick skeletal muscle after blockage of old receptors with unlabelled α-bungarotoxin. Unlabelled α-bungarotoxin was removed and inhibitors added at t = 0. Number of newly incorporated receptors has been normalized to total number of original surface receptors at t = 0. Data are from 5 experiments for cycloheximide, 3 for puromycin, 2 for 2,4-dinitrophenol, 1 for each temperature. Data for each individual experiment have been normalized so that the control rate is 4.5%/h. Actual control rates ranged from 3.8%/h to 5.1%/h. (From Fambrough et al. 1977).

Most extrajunctional ACh receptor molecules are mobile in the plasma membrane (Axelrod et al., 1976). However, clustered extrajunctional ACh receptors and also synaptic receptors are stationary on a time-scale of minutes to hours (Axelrod et al., 1976; Fambrough and Pagano, 1977). It is unknown whether the mechanisms limiting receptor mobility in these two cases are the same.

DEGRADATION

Average extrajunctional receptor lifetimes have been estimated as 8–30 h for ACh receptors in various muscles and muscle cultures from homeothermic species. For cultured chick (Gardner and Fambrough, 1978) and calf (Merlie et al., 1976) skeletal muscle in vitro, receptor lifetimes (approximately 18 h) have been determined by pulse-chase experiments involving direct labeling of receptor molecules with isotopically labelled amino acids. In other cases, estimates are based upon the loss of radioactivity from the muscle after iodinated α-bungarotoxin was bound to ACh receptor sites. The bound α-bungarotoxin may slightly decrease the turnover rate (Fig. 5).

Studies on the mechanism of degradation have, for the most part, involved labelling of receptor sites with iodinated α-bungarotoxin and then following the

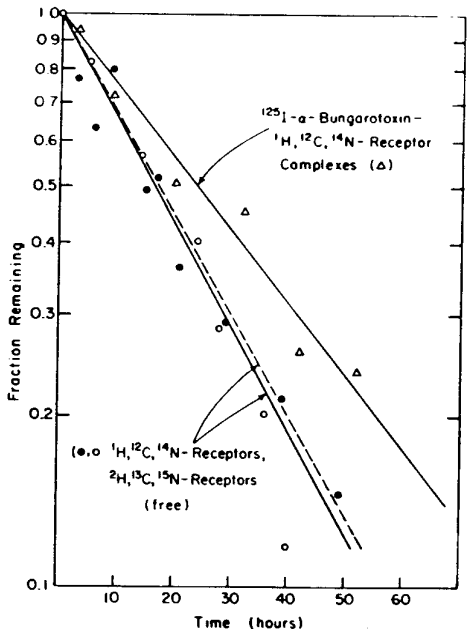

Fig. 5. Degradation of normal and density shifted receptors without bound α-bungarotoxin, employing a pulse chase experimental regime. The open and closed circles represent two experiments combining the degradation data for ^{1}H-, ^{12}C-, ^{14}N-receptors and ^{2}H-, ^{13}C-, ^{15}N-receptors from the pulse and chase phase of the experiments respectively. The results of one parallel experiment measuring the decay of ^{125}I-α-bungarotoxin receptor complexes from an identically treated set of cultures is also shown (△——△). (From Gardner and Fambrough, 1978).

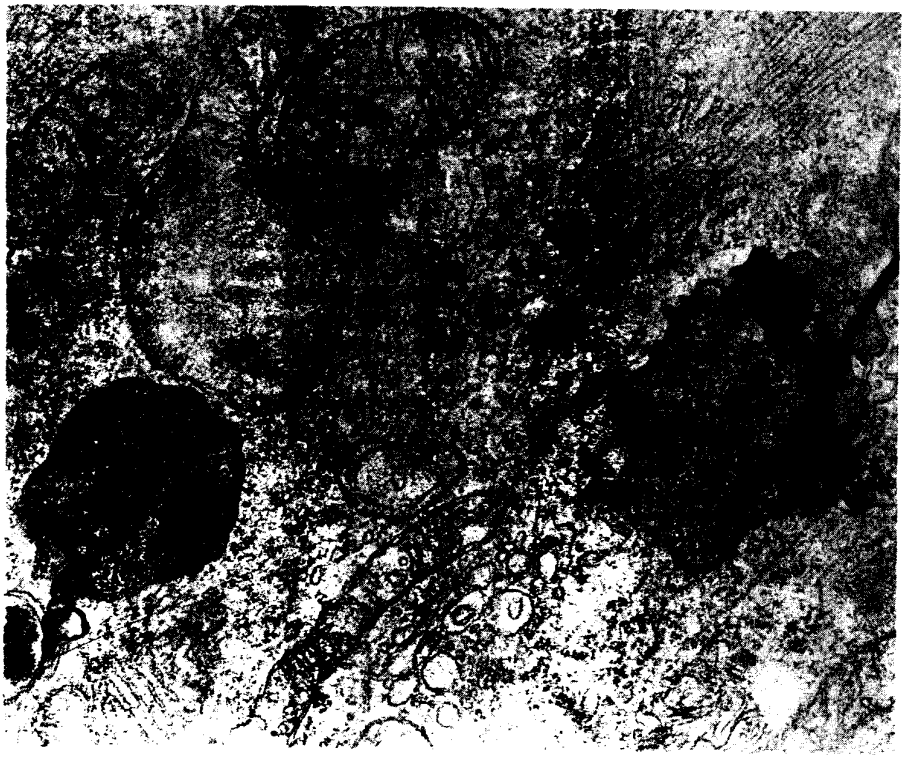

Fig. 6. Electron microscope autoradiogram showing silver grains over secondary lysosomes of cultured chick muscle. Cells were first labelled with ^{125}I-α-bungarotoxin, fixed in glutaraldehyde, and prepared for EM autoradiography. The appearance of grains over secondary lysosomes is interpreted as evidence for the uptake of bungarotoxin receptor complexes into the cytoplasm, and their intracellular degradation. (From Devreotes and Fambrough, 1976a).

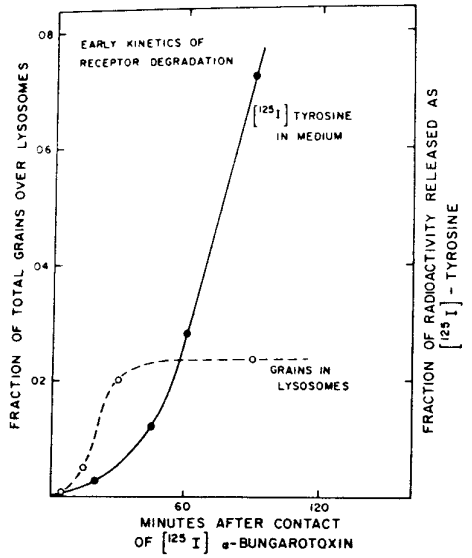

Fig. 7. Kinetics of transport of radioactivity to secondary lysosomes (open circles) and kinetics of liberation of iodo-tyrosine from chick muscle cultures (solid circles) after brief exposure of muscle to ^{125}I-α-bungarotoxin. Data obtained from electron microscopic autoradiograms and from chromatographic analysis of culture medium. (From Fambrough et al., 1977).

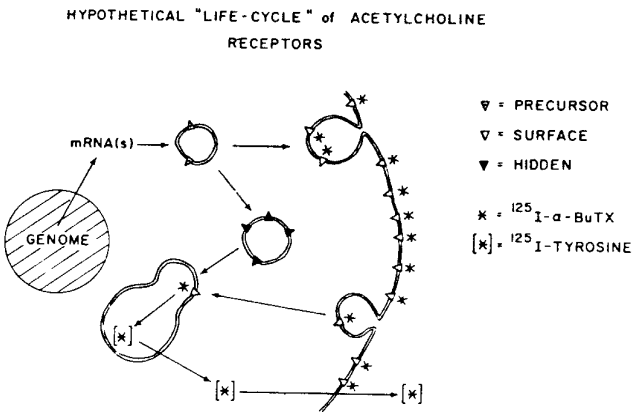

Fig. 8. Schematic life history of ACh receptors in cultured skeletal muscle. The figure depicts a cross section through a myotube with ACh receptors symbolized as triangles in membrane profiles. The newly synthesized receptors (▽) occur in Golgi apparatus and post-Golgi vesicles and constitute about 10–15% of total ACh receptors. ACh receptors in the plasma membrane (▽) are depicted as associated with iodinated α-bungarotoxin (*) as would occur in an experiment to estimate turnover rate. Iodotyrosine, the radioactive product of proteolysis of the iodinated α-bungarotoxin, is symbolized by [*]. About 70% of the ACh receptors are located in the plasma membrane as individual molecules and aggregates. Only about 2% of receptors are in the degradation pathway at any instant. The remaining ACh receptors (▼) occur on internal membranes and have been referred to as "hidden" because they are not labelled by extracellular α-bungarotoxin. The average lifetime of the hidden sites is about the same as for receptors in the plasma membrane.

fate of the radioactivity (Berg and Hall, 1974; Chang and Huang, 1975; Devreotes and Fambrough, 1975, 1976a). The degradation process is energy-dependent and not tightly coupled to receptor biosynthesis. α-Bungarotoxin-receptor complexes are internalized and transported to secondary lysosomes (Fig. 6) where proteolysis occurs. Proteolytic breakdown can be blocked by long-term treatment of muscle with Trypan blue. Treated cells can still internalize receptors and transport them to lysosomes, but breakdown does not occur and radioactivity accumulates to high levels in the lysosomal compartment (over 20% in 8 h). The kinetics of transport to lysosomes have been estimated roughly by electron microscope autoradiography (Fambrough et al., 1977), and they seem to be consistent with other kinetic data on receptor turnover (Fig. 7).

Figure 8 is a cartoon illustrating the main features of ACh receptor metabolism. The biosynthetic route is essentially that used in other cells for secretion of secretory proteins. This is probably true for muscle as well. Rotundo (Rotundo and Fambrough, 1977, and unpublished observations) has studied the kinetics of secretion of ACh esterase in detail and proposed that secretion of this glycoprotein and the biogenesis of plasma membrane involve the same mechanism in skeletal muscle. Thus any perturbation of ACh receptor biosynthesis, transport and incorporation into plasma membrane is accompanied by an equal perturbation of ACh esterase biosynthesis and secretion.

CONTROL OF EXTRAJUNCTIONAL ACh RECEPTORS

The appearance of ACh hypersensitivity following denervation of adult skeletal muscle has been shown to result from turn-on of receptor biosynthesis (Brockes and Hall, 1975; Devreotes and Fambrough, 1976b). Just which aspect of the denervated state triggers renewed receptor synthesis is not known. However, it is clear that this turn-on can be reversed by direct stimulation of denervated muscle (Lømo and Rosenthal, 1972). Electrical stimulation of hypersensitive muscle in organ culture suppresses the biosynthesis and incorporation of receptor molecules into extrajunctional plasma membrane (Reiness and Hall, 1977; Card, unpublished observations, Fig. 9), while somewhat slowing (Hogan et al., 1976) or not changing (Card, 1977) the rate of extrajunctional ACh receptor degradation. Thus the disappearance of hypersensitivity during electrical stimulation results from decline in the number of extrajunctional ACh receptors due to degradation without balancing replacement. The mechanism underlying the depression of ACh receptor biosynthesis remains unknown.

FOR THE FUTURE

In the context of cholinergic mechanisms, the eventual goals in the study of receptor life history are to describe the mechanisms and the rates of production and destruction of receptors in developing and adult muscle and to determine all of the mechanisms regulating metabolic rates and spatial distribution of recep-

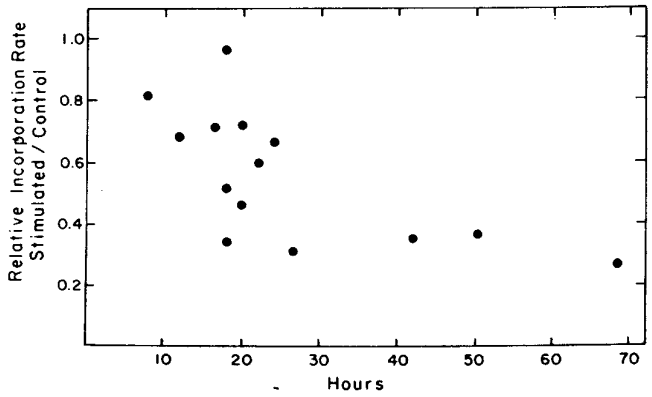

Fig. 9. Effect of electrical stimulation on biosynthesis and plasma membrane incorporation of ^{2}H-, ^{13}C-, ^{15}N-labelled ACh receptors in denervated rat extensor digitorum longus muscle in organ culture. These muscles contracted vigorously with full tetanic tension, in response to the electrical stimulation (100 Hz for 1 sec given every 80 sec). Relative biosynthetic rate was measured as the amount of ^{2}H-, ^{13}C-, ^{15}N-containing receptors (identified by velocity sedimentation sucrose gradients) per mg muscle weight in stimulated muscles divided by that measured in non-stimulated muscles cultured for the same time. Cultures were labelled with ^{2}H-, ^{13}C-, ^{15}N-amino acids for 7–9 h in experiments where stimulation was for 8–18 h. All longer experiments had labelling times of 11–12 h. Each point on the graph represents one experiment, involving two or three stimulated muscles and two or three control, non-stimulated muscles.

tor molecules. One of tne most elusive goals continues to be the assessment of the roles of factors other than muscle activity in regulation of receptor metabolism.

Several recent observations may prove pivotal to further progress. Apparently medium components derived from nervous tissue can alter the number and distribution of ACh receptors (Betz and Osborne, 1977; Cohen and Fischbach, 1977; Christian et al., 1978; Younkin et al., 1978). Receptor distribution is also sensitive to electric fields (Orinda and Poo, 1978). And receptor biosynthesis is accelerated by some drugs which may affect membrane structure and function (Shainberg et al., 1976). There is a promising variety of muscle and nerve-muscle preparations which appear favorable for further biophysical, biochemical and genetic analysis of receptor distribution, metabolism and regulation.

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