gen is involved in human ear diseases is not known. Preliminary studies performed in our laboratory suggest that 50 percent of patients with Ménière's disease have collagen autoimmunity (*15*). On this basis, we propose that the hydrops may be initiated by autoimmunity to collagen. Our animal model may thus be useful in defining the pathogenesis of human Ménière's disease.

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Rapid Degradation of "New" Acetylcholine Receptors at Neuromuscular Junctions

Abstract. *Acetylcholine receptors at innervated neuromuscular junctions are very stable, with half-lives reported to be 6 to 13 days. Their turnover is described as a first-order process, implying a single population of receptors. In this study, two subpopulations of acetylcholine receptors at normally innervated junctions have been identified. One has a rapid turnover rate with a half-life of 18.7 hours, similar to that of extrajunctional receptors, and the other has a slow turnover rate with a half-life of 12.4 days. The rapidly turned over subpopulation represents approximately 20 percent of the total junctional receptors. This finding may account for the discrepancies in previous reports of turnover rates and may explain the rapid reversibility in vivo of agents that "irreversibly" block acetylcholine receptors. This finding also implies that the synthesis rate of junctional acetylcholine receptors may be higher than previous estimates. The rapidly turned-over subpopulation may represent receptors that were newly inserted into the neuromuscular junction and that were not yet stabilized by an influence of the motor nerve.*

Acetylcholine (ACh) receptors at normally innervated neuromuscular junctions are very stable, in contrast to the ACh receptors at extrajunctional regions of denervated muscle (*1, 2*). The turnover of extrajunctional ACh receptors has been described as a single-order process (*3*); the receptors are rapidly degraded, with a half-life of 15 to 30 hours (*4*). Junctional ACh receptors have a far slower rate of degradation, also described as a single-order process, with a half-life of 6 to 13 days (*2, 3, 5–7*). However, scrutiny of the data in several earlier studies suggested that a subpopulation of the junctional receptors may be degraded more rapidly (*3, 5, 8*). We examined the turnover of ACh receptors at intact neuromuscular junctions and now report that a sizable fraction of junctional receptors have a fast turnover rate. This population of ACh receptors may represent newly inserted ACh receptors that have not yet been stabilized at the postjunctional membrane.

We determined the rate of ACh receptor degradation by monitoring the loss of ^{125}I-labeled α-bungarotoxin (^{125}I-α-BuTx) that was specifically bound to junctional ACh receptors (*3, 9*). The loss of bound ^{125}I-α-BuTx was found to correspond to the loss of ACh receptor sites (*9*). The ^{125}I-α-BuTx is degraded along with the ACh receptor and is released from the muscle in the form of ^{125}I-tyrosine (*3, 6, 9*).

Binding of ^{125}I-labeled or unlabeled α-BuTx to ACh receptors of the mouse diaphragm was carried out as described (*6, 10*). Adult female (C57BL/6 × DBA/2)F_1 hybrid mice (18 to 20 g) were anesthetized with chloral hydrate (0.4 mg per gram of body weight) for all surgical procedures. Mice were given unlabeled α-BuTx (1 μg per 20 g of body weight) or ^{125}I-α-BuTx (1.4 μg per 20 g of body weight; specific activity, 3.91×10^4 to 7.96×10^4 Ci/mole) in 140 μl of Ringer solution; half the dose was injected into each thoracic cavity. The mice were then maintained in an upright position for 1 to 2 hours to allow the solution to gravitate to the diaphragm and block or label the ACh receptors.

At various times after the blocking and labeling procedures, diaphragms were removed from groups of three or four mice. Care was taken to account for any background radioactivity (due to radioactive material diffusely bound along the muscle membrane). First, the diaphragms, in groups of three or four, were washed repeatedly for 48 hours with large volumes of buffered Ringer solution until no further radioactivity was detected in the final wash. Second, correction was made for background radioactivity in the junction-containing strip. For this purpose, the diaphragm was cut into junction-containing and extrajunctional strips. The radioactivity measured in the extrajunctional strip was subtracted (on a per-weight basis) from the radioactivity in the junction-containing strip. At all time points, this background radioactivity was a small fraction of the total radioactivity in the junctional portion of the muscle; at its highest, 1 day after the labeling procedure, background radioactivity was less than 10 percent of the total counts. To evaluate the contribution of the perijunctional regions to the turnover of receptors, we also used quantitative light autoradiography to examine the distribution of ^{125}I-α-BuTx binding (*11*).

The radioactive material remaining bound to the neuromuscular junctions was determined for groups of mice at each time point. Because it takes several hours for binding of ^{125}I-α-BuTx and washout of unbound ^{125}I-α-BuTx to occur in vivo, measurements were begun 1 day after the labeling procedure. All subsequent counts were expressed as a percentage of the total counts present at day 1 after labeling, and the means were plotted on a logarithmic scale against time. Straight lines were fitted to the points by the method of least squares, and half-lives of ACh receptors were calculated from the slopes of the lines.

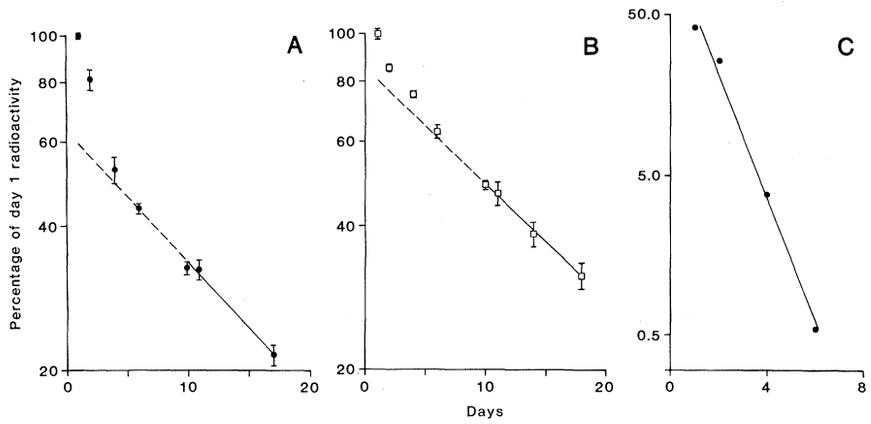

Fig. 1. (A) Loss of radioactivity from mouse diaphragms after labeling of newly inserted ACh receptors with ^{125}I-α-BuTx. Six days before the labeling procedure, existing ACh receptors were blocked with unlabeled α-BuTx. On day 0, receptors that had been newly inserted into the muscle membrane were labeled with ^{125}I-α-BuTx. At intervals of 1 to 17 days thereafter, diaphragms were removed and the radioactivity associated with binding to the junctional region was determined. Each point is the mean ± standard error for 3 to 11 diaphragms and represents the percentage of the radioactive material bound at day 1 plotted on a logarithmic scale against days after labeling. The dashed line is extrapolated back from the regression line fitted to the data from day 10 to 17. (B) Loss of radioactivity from diaphragms after labeling of ACh receptors with ^{125}I-α-BuTx on day 0, without previous treatment. Each point is the mean ± the standard error for 3 to 15 diaphragms. The dashed line is extrapolated back from the regression line fitted to the data from day 10 to 18. (C) Loss of radioactivity associated with the subpopulation of junctional ACh receptors with a rapid rate of degradation (see text).

We initially examined the turnover of ACh receptors that had been newly inserted into the neuromuscular junction of the mouse diaphragm. The preexisting ACh receptors were first blocked in vivo with unlabeled α-BuTx by injecting the toxin into the thoracic cavity. After an interval of 6 days to permit the synthesis and insertion of a pool with a relatively high proportion of newly inserted ACh receptors, the receptors were labeled in vivo with ^{125}I-α-BuTx. Groups of mice were killed at intervals of 1 to 17 days thereafter. The diaphragms were removed and washed, and the radioactivity associated with specific binding to junctional regions was determined as described above. Three separate experiments on a total of 49 mice were pooled and showed that the $\log_{10}$ plots of loss of radioactivity could be roughly approximated by two straight lines (Fig. 1A). From day 1 to day 4, radioactivity was lost with an apparent half-life of 3.2 days. By contrast, from day 10 to day 17, radioactivity was lost at a rate that was significantly slower ($P < 0.01$), with a half-life of 11.0 days.

To determine whether the initial rapid loss of radioactivity reflected a subpopulation of receptors that occur naturally at neuromuscular junctions (that is, without prior α-BuTx treatment), we determined the rate of receptor turnover in previously untreated diaphragms and searched for a small proportion of rapidly degraded receptors. Acetylcholine receptors were labeled with ^{125}I-α-BuTx; at 1 to 18 days thereafter, diaphragms were removed, and the specific binding to the junctional strip was determined as above. The pattern of loss of radioactivity was examined in four separate experiments and pooled (Fig. 1B). From day 1 to day 2 after labeling, radioactivity was lost with an apparent half-life of 4.3 days. This rate was significantly faster than the rate of loss during the interval from 10 to 18 days, which corresponded to a half-life of 12.4 days ($P < 0.01$). These findings confirmed at least two rates of loss of radioactivity in the junctional regions of previously untreated diaphragms.

Since this study was principally concerned with the identification of a population of rapidly degraded ACh receptors at neuromuscular junctions, it was important to eliminate artifacts due to (i) washout of ^{125}I-α-BuTx nonspecifically trapped in the muscle and (ii) rapid turnover of nonjunctional ACh receptors. In the first instance, we washed the diaphragms for more than 48 hours after removal from the animals, by which time virtually no radioactivity appeared in the wash medium. In the second instance, we corrected for the small amount of background ^{125}I-α-BuTx at the junctional region by subtracting the amount of radioactivity associated with an extrajunctional strip of the muscle of equal size. Thus, loss of radioactivity from the junctional regions most probably represents only receptor degradation. Finally, we used quantitative autoradiography to examine the possibility that the rapidly turned-over ACh receptors might be perijunctional rather than junctional. Grain counts of autoradiograms showed that the perijunctional regions bound only a small amount of ^{125}I-α-BuTx (after subtraction of background) and that the grains in the perijunctional region did not decline disproportionately rapidly after labeling (*12*). These findings are consistent with earlier reports that the ACh receptors are highly localized at the neuromuscular junctions (*13*) and indicate that the radioactivity that is lost rapidly in the above two experiments is associated with ^{125}I-α-BuTx binding to the junctional region.

Our experiments indicate that two subpopulations of receptors, differing in their rates of turnover, exist at intact neuromuscular junctions. An estimate of the fraction of receptors that turn over slowly at the normal neuromuscular junction can be obtained by extrapolating the slower degradation rate—the rate from day 10 to day 17—back to day 1. The data in Fig. 1B indicate that 81 percent of the total population of receptors is degraded slowly. Thus the rapidly turned-over subpopulation represents about 20 percent of the total. The apparent half-life of this subpopulation is 3.5 days. However, this apparent rate represents a mixture of the degradation rate of a subpopulation of rapidly degraded receptors superimposed on a much larger population of slowly degraded receptors. We can estimate the turnover rate of the rapidly degraded population alone by subtracting the rate for the slowly degraded population from the total. Thus, in Fig. 1A, the slow rate of loss of radioactivity is extrapolated back to day 1 (dashed line). At each time point from day 1 to day 6, the calculated fraction of radioactivity associated with the slowly degraded receptors is subtracted from the total radioactivity. The remainder represents an estimate of the radioactivity associated with the rapidly degraded ACh receptors, and the $\log_{10}$ of this value is plotted against time in Fig. 1C. These data give a good fit to a straight line (correlation coefficient, 0.995; $P < 0.005$), with a half-life of 18.7 hours. This degradation rate is similar to that reported for extrajunctional ACh receptors in the mouse diaphragm (*10*) and in other preparations (*4*). Since newly inserted receptors are degraded at a rapid rate, the rate of receptor synthesis must also be faster than had been previously estimated, to maintain a steady state. It appears therefore that this subpopulation of ACh receptors at the neuromuscular junction has at least one property in common with extrajunctional ACh receptors, a rapid rate of turnover. It remains to be determined whether the

newly inserted receptors share other properties of extrajunctional receptors.

The finding of a subpopulation of rapidly degraded ACh receptors in the junctional region may shed light on certain puzzling observations. First, the observation that neuromuscular transmission recovers more quickly than expected after blockade of ACh receptors with the irreversible agent α-BuTx (*3, 14*) might be explained by the more rapid rate of junctional receptor synthesis predicted by our results; a proportion of the blocked ACh receptors would thus be quickly replaced by newly synthesized receptors. Second, the rapid degradation of a subpopulation of receptors may help to explain discrepancies in previous estimates of junctional ACh receptor turnover rates; estimates of half-lives of junctional ACh receptors range from 6 to 13 days. Measurements of receptor loss begun soon after labeling and continued for only a short period would give an apparently faster rate of junctional ACh receptor turnover and a correspondingly shorter half-life than measurements begun later after labeling, or for longer periods, when the subpopulation of rapidly degraded receptors would have less influence on the overall receptor degradation rate. For example, in an earlier study we had examined degradation occurring for a relatively short time and found a half-life of 5.6 days for junctional ACh receptors (*6*) as compared with the half-life of 12.4 days obtained in the present study in which turnover was examined later after labeling and for a longer time period.

The coexistence of rapidly and slowly degraded ACh receptors at the end plate can be explained by two hypotheses. First, it is possible that ACh receptors are initially inserted into the postsynaptic membrane in a form that is relatively unstable and that they are subsequently stabilized by some influence of the motor nerve. Alternatively, there may be two or more independent pools of ACh receptors with different intrinsic turnover rates. Earlier observations that the slow degradation rate of preexisting junctional ACh receptors can be increased after denervation indicate that junctional ACh receptors can be destabilized by removing the influence of the motor nerve (*7, 10*). Furthermore, ACh receptors that are inserted into the junctional region after a period of denervation have a turnover rate that is very rapid and is similar to that of extrajunctional ACh receptors (*15*). Taken together, these observations favor the nerve stabilization hypothesis. Reiness and Weinberg (*16*) have shown that receptors in new ectopic synapses have a rapid rate of turnover and may correspond to the rapidly degraded receptors identified in this study at the intact, mature neuromuscular junction. How the nerve might influence the stability of ACh receptors at the neuromuscular junction remains to be determined. It could act directly by altering the ACh receptors or their immediate environment or indirectly by inducing a change in the postsynaptic membrane or underlying cytoskeleton of the muscle cell.

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11. The location of ^{125}I-α-BuTx binding sites in the junctional and perijunctional region was determined by light autoradiography. At 1, 6, and 15 days after ^{125}I-α-BuTx labeling in vivo, diaphragms were removed, washed, fixed in 2 percent glutaraldehyde, washed in phosphate buffer, and teased into single fibers on gelatin-coated slides in a drop of 5 percent albumen [S. Burden, *Dev. Biol.* **57**, 317 (1977)]. The slides were dipped in Kodak emulsion NBT2, allowed to dry, and left to expose for 24 hours at room temperature. Grains in the junctional and perijunctional regions were counted as follows. The muscle fiber was positioned under the microscope at ×100 so that a 50-μm square grid in the eyepiece was located over the densest accumulation of grains. This was defined as the junction-containing strip of the muscle fiber. The 50-μm strips on either side of this region were defined as the perijunctional strips. Background binding was determined both from the slide in a region away from the muscle fibers and from single, teased fibers from an extrajunctional segment of the diaphragm. Grain counts on the extrajunctional muscle fibers were not significantly higher than counts on an equivalent area of the slide. All grains on the muscle fiber and within 5 μm on either side of the fiber were included in the counts, and background counts were subtracted. One day after labeling a mean ± standard deviation of 247 ± 95 grains (N = 23 fibers) were counted in the 50-μm junctional strip, and a total of 13.0 ± 11.3 grains were counted in the two 50-μm perijunctional strips.
12. The perijunctional strips contained only 2.5 percent as many grains as were present in the junctional strip (N = 23 fibers). The percentage in the perijunctional region did not decline at 6 days (4 percent, N = 16), nor 15 days (3.5 percent, N = 6) after labeling. Thus, during the first few days after labeling, when most of the rapid rate of loss of radioactivity occurred, there was no decrease in the proportion of perijunctionally bound ^{125}I-α-BuTx. Furthermore, when diaphragms were labeled 6 days after existing receptors were blocked with unlabeled α-BuTx, there was no greater percentage of grains in the perijunctional region (2. 6 percent, N = 15).
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Intracellular Recordings from Cochlear Inner Hair Cells: Effects of Stimulation of the Crossed Olivocochlear Efferents

Abstract. *Intracellular recordings were obtained from inner hair cells located in the lower basal turn of the guinea pig cochlea. At low sound pressure levels the inner hair cells were highly frequency selective, producing receptor potentials only in response to sound frequencies between about 16 and 24 kilohertz. Electrical stimulation of efferent nerves in the crossed olivocochlear bundle markedly reduced these receptor potentials while causing little change in the resting membrane potential. At high sound levels, where cells responded to an increasingly wider range of sound frequencies, stimulation was less effective in reducing receptor potentials. Since the crossed olivocochlear bundle primarily innervates outer hair cells, these results support an outer hair cell contribution to the most sensitive response region of inner hair cells.*

Octavo-lateralis sensory systems are characterized by an abundant efferent, as well as afferent, innervation (*1*). In the mammalian cochlea, the two types of sensory receptors, or hair cells, receive distinct patterns of innervation (Fig. 1A). Whereas inner hair cells (IHC's) receive the bulk (85 to 95 percent) of the afferent innervation (*2*), in the guinea pig, efferent endings on IHC's occur only rarely (*3*). Outer hair cells (OHC's) receive a large number of efferent endings, many of which are from axons of the crossed olivocochlear bundle (COCB), which originates in the brainstem in the medial portion of the superior olivary complex