CC/NUMBER 37
SEPTEMBER 10, 1990

This Week's Citation Classic®

Hall Z W. Multiple forms of acetylcholinesterase and their distribution in endplate and non-endplate regions of rat diaphragm muscle. *J. Neurobiology* 4:343-61, 1973.
[Department of Neurobiology, Harvard Medical School, Boston, MA]

Three species of acetylcholinesterase were identified in rat diaphragm muscle by sucrose gradient sedimentation. Two of them were found throughout the muscle, but the third was only present in regions of muscle containing endplates. This form was not present in nerve and was almost completely lost after denervation. [The *SCI*® indicates that this paper has been cited in over 385 publications, making it the most-cited paper from this journal.]

Endplate Acetylcholinesterase in Muscle

Zach W. Hall
Department of Physiology
University of California
San Francisco, CA 94143-0444

May 11, 1990

When I wrote this paper, I was a junior faculty member in the Department of Neurobiology at Harvard Medical School, where I had been recently joined by Regis Kelly, a colleague whom I had met when we were postdoctoral fellows in biochemistry at Stanford. At Harvard, we were trying to develop a biochemical approach to the neuromuscular junction, which at that time was the synapse whose electrophysiological function was best understood. The important role of acetylcholinesterase at the junction was well known. Histochemical experiments by Couteaux and others had shown it to be highly concentrated there, and electrophysiological experiments demonstrated that it acted to terminate the action of the transmitter, acetylcholine, on the postsynaptic membrane.

Our experiments began with an accidental finding. We were trying to purify the surface membranes of muscle fibers and were using acetylcholinesterase, which we assumed to be an integral membrane protein, as a marker for the postsynaptic membrane. To remove contaminating connective tissue, we treated a partially purified surface membrane preparation with collagenase. To our surprise we found that this treatment solubilized acetylcholinesterase from the membranes. We repeated the experiment on intact muscle and found that incubation with collagenase released active enzyme from the muscle into the medium without affecting the physiological properties of the muscle fiber membrane. Physiological and histochemical experiments showed that the released acetylcholinesterase came from the endplate. Moreover, it came only from the endplate; acetylcholinesterase in parts of the muscle lacking endplates was unaffected by collagenase treatment. These results, which we published in *Nature*,[1] suggested to us not only that the endplate enzyme was associated with extracellular matrix material, but also that it must be different from the rest of the acetylcholinesterase in the muscle.

I then attempted to extract and purify acetylcholinesterase from muscle to see if I could find a distinctive endplate form. Conventional biochemical methods did not seem to work, however. The enzyme aggregated easily, and, using a variety of column procedures, I was unable to obtain discrete, reproducible peaks of activity. Two key ideas broke the logjam. First, Kelly suggested analysis by sucrose gradient sedimentation. This method gave two distinct fractions, but showed no difference between regions of muscle with endplates and those without. Then, I found a paper in French by J. Massoulié and F. Rieger on acetylcholinesterase from the electric organ of eel.[2] With the aid of a dictionary, I laboriously translated this paper word-by-word into an English version, which I still have. The effort was worth it. This paper, along with several others from Massoulié's laboratory, reported that eel electric organ contained a species of acetylcholinesterase that was highly asymmetric and that aggregated at low salt concentrations, but was soluble in 1M NaCl. Application of the high salt method to muscle immediately revealed a new sucrose gradient peak that corresponded to the asymmetric form and that was found only in endplate regions of muscle. These were the results reported in the *Journal of Neurobiology* article.

Taken together, the results of our two papers suggested that a special form of acetylcholinesterase is present at endplates and that it is not a membrane protein, but is associated with the extracellular matrix, perhaps by a mechanism involving collagen. Subsequent work by I. Silman[3] and by P. Taylor[4] on acetylcholinesterase from electric fish showed that the asymmetric form indeed has a special structure in which three tetramers of catalytic subunit are attached to a long collagen-like tail. U.J. McMahan and coworkers[5] then demonstrated directly that acetylcholinesterase at the neuromuscular junction is part of the basal lamina, presumably attached by the tail. The *Journal of Neurobiology* paper thus formed an initial link in the continuing investigation of the relation between the physiological function and molecular structure of synaptic acetylcholinesterase.[6]

1. **Hall Z W & Kelly R B.** Enzymatic detachment of endplate acetylcholinesterase from muscle. *Nature* 232:62, 1971. (Cited 195 times.)
2. **Massoulié J & Rieger F.** L'acetylcholinesterase des organes electriques de poisson (torpille et gymnote); complexes membranaires (The acetylcholinesterase of the electric organs of fish (numb-fish and electric eel); complex membranes). *Eur. J. Biochem.* 11:441-55, 1969. (Cited 185 times.)
3. **Anglister L & Silman I.** Molecular structure of elongated forms of electric eel acetylcholinesterase. *J. Mol. Biol.* 125:293-311, 1978. (Cited 70 times.)
4. **Lwebuga-Mukasa J S, Lappi S & Taylor P.** Molecular forms of acetylcholinesterase from *Torpedo californica*: their relationship to synaptic membranes. *Biochemistry—USA* 15:1425-34, 1976. (Cited 140 times.)
5. **McMahan U J, Sanes J R & Marshall L M.** Cholinesterase is associated with the basal lamina at the neuromuscular junction. *Nature* 271:172-4, 1978. (Cited 210 times.)
6. **Taylor P, Schumacher M, MacPhee-Quigley K, Friedmann T & Taylor S.** The structure of acetylcholinesterase: relationship to its function and cellular disposition. *Trends Neurosci.* 10:93-6, 1987. (Cited 15 times.)

IA-20