Cholinesterase in a Receptor

Abstract. Cholinesterase is found at the sites for mechanoelectric conversion of Pacinian corpuscles; its acetylcholine-splitting activity is of the same order of magnitude as that of the cholinesterase at neuromuscular junctions. The enzyme is not a typical acetylcholinesterase.

When a Pacinian corpuscle is pressed upon, a graded potential (generator potential) is produced inside the sense organ. Within certain limits, this potential is a linear function of the applied pressure. Although it is now clear that the generator potential is produced at the nonmyelinated nerve ending inside the corpuscle (*1*), nothing is known about the mechanisms which convert the deformation into the generator potential. From time to time it has been surmised that a substance like acetylcholine might be involved in the excitation of this and other receptors. In fact, a variety of mechanoreceptors are sensitive to applied acetylcholine (*2, 3*). More direct evidence for participation of acetylcholine in reception, such as evidence that acetylcholine is present in the receptor, is lacking.

An early attempt to test for the presence of acetylcholine in Pacinian corpuscles gave negative results (*2*). There are, however, a considerable number of difficulties encountered in assaying acetylcholine in the corpuscle: the esterase is also likely to be present and, in addition, there are strong diffusion barriers (*4*). Any attempt to block the esterase by applying antiesterases from within or without the sense organ involves dealing with the diffusion barriers of multiple lamellae inside the capsule. An assay of the sense organ's cholinesterase, which is a very stable enzyme, seemed more promising. Although this may seem a priori a more indirect approach, the presence of a particular enzyme, namely acetylcholinesterase, in a tissue is in general a good indication that the corresponding substrate is there metabolized. This report deals with the nature, localization, and concentration of cholinesterase in the Pacinian corpuscle (*5*).

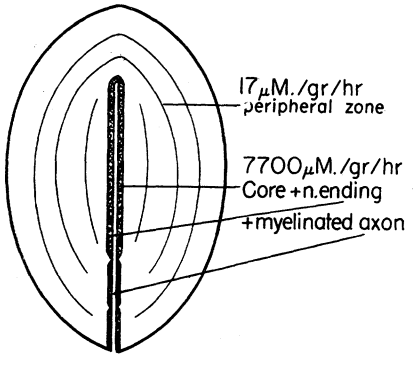

Fig. 1. Distribution of cholinesterase in a Pacinian corpuscle. The figures to the right indicate the amount of acetylcholine which is split in 1 hour by 1 g of the corresponding tissue of the sense organ. Note the very high cholinesterase activity around the nerve ending; this activity is of the same order of magnitude as that of a motor end plate of frog sartorius muscle.

Single corpuscles were obtained from the mesentery and the pancreas of the cat. They were freed by dissection from mesenteric and pancreatic tissue, and their axon was cut at the point where it emerges from the corpuscle. From 50 to 600 corpuscles, depending on the kind of experiment to be performed, were thus prepared for each assay. They were kept and homogenized in a refrigerated Krebs's solution. The cholinesterase activity of the homogenates was determined by Hestrin's technique (*6*). A few determinations were also made by the usual manometric technique; they gave essentially the same results.

Pacinian corpuscles were found to contain rather large amounts of cholinesterase. As averaged over 1060 corpuscles, a concentration of cholinesterase capable of splitting 16 mg of acetylcholine per hour, per gram of corpuscle tissue, was found. A single corpuscle of normal size contains a concentration of cholinesterase which hydrolyzes 1.6 μg of acetylcholine per hour. The enzyme in the corpuscle is not a typical acetylcholinesterase, for its rate of hydrolysis of butyrylcholine is approximately three times that of acetylcholine (at concentrations of from 4 to 5 μmole per milliliter of substrate known to be optimal for acetylcholine). Besides, the esterase does not show the clear optimum of substrate (acetylcholine) concentration which is characteristic of acetylcholinesterase (*7*). Instead, no sign of inhibition of enzyme activity is observed even with substrate concentrations raised to 0.1*M*, at which concentrations the activity of acetylcholinesterase would have become considerably depressed. On the other hand, the enzyme does not split any detectable amount of monobutyrine. It is therefore a cholinesterase, although not of the typical kind of acetylcholinesterase found in many excitable tissues (*7, 8*).

Our most interesting observation was the uneven distribution of cholinesterase inside the structures of the corpuscle. By far the largest part of the enzyme appeared to be localized in the axon and ending, and in the thin hull of core structure which surrounds the ending. The remaining tissue of the corpuscle contains only little of the enzyme (Fig. 1). In these experiments, single corpuscles were prepared as described above, but, in addition, the "peripheral zone" of each corpuscle was separated by dissection from the "inner core" (*1*), which contains the nerve ending and has a stump of about 200 μ of myelinated axon attached to the latter. Both fractions of the sense organ were separately homogenized and tested for cholinesterase. It was found that the core fraction—that is, the nerve ending plus the myelinated stump plus the core—contains cholinesterase capable of splitting 1390 mg of acetylcholine per hour per gram. This provides the structures around the nerve ending with the ability to hydrolyze 0.7×10^9 molecules of acetylcholine per millisecond—a figure similar to that calculated (1.6×10^9) for a single end plate of frog sartorious muscle (*7*). The capsular fraction, on the other hand, which amounts to more than 99 percent of the corpuscle's mass, splits as little as 3 mg of acetylcholine per hour per gram.

Since the cholinesterase found is not a typical acetylcholinesterase, the question of the nature and function of the enzyme and its normal substrate remains to be elucidated. It is noteworthy, however, that the high concentrations of cholinesterase were found precisely around the nonmyelinated nerve ending, where also the generation of electric activity of the receptor has been shown to take place (*1*), whereas, on the other hand, the large peripheral capsular zone, which does not participate in mechanoelectric conversion (*1*), was found to contain only a negligible amount of the enzyme.

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References and Notes

1. W. R. Loewenstein and R. Rathkamp, *J. Gen. Physiol.* 41, 1245 (1958).
2. G. L. Brown and J. A. B. Gray, *J. Physiol. (London)* 107, 306 (1948).
3. R. Granit, S. Skoglund, S. Thesleff, *Acta Physiol. Scand.* 28, 134 (1953); C. A. G. Wiersma, E. Furshpan, E. Florey, *J. Exptl. Biol.* 30, 136 (1953); S. Landgren, G. Liljenstrand, Y. Zotterman, *Acta Physiol. Scand.* 30, 105 (1954); A. S. Paintal, *J. Physiol. (London)* 126, 271 (1954); J. Diamond, *ibid.* 130, 513 (1955); W. R. Loewenstein, *ibid.* 132, 40 (1956); A. S. Jarret, *ibid.* 133, 243 (1956).
4. J. A. B. Gray and M. Sato, *J. Physiol. (London)* 129, 594 (1955).
5. The work reported in this study was supported by research grants from the National Institutes of Health, U.S. Public Health Service (B-1466-R1); the National Cystic Fibrosis Research Foundation; and the National Science Foundation. We take pleasure in thanking Dr. D. Nachmansohn of the biochemistry department, Columbia University, College of Physicians and Surgeons, for valuable suggestions and help.
6. S. Hestrin, *J. Biol. Chem.* 180, 249 (1949).
7. D. Nachmansohn, *Ergeb. Physiol. u. exptl. Pharmakol.* 48, 575 (1955).
8. R. H. S. Thompson, *Brit. Med. Bull.* 9, 138 (1953); G. B. Koelle, *J. Comp. Neurol.* 100, 211 (1954).

30 June 1958