THE METABOLISM OF ACETYLCHOLINE

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In discussing the metabolism of acetylcholine (ACh) in sensory nerves, we must start with the presence of ACh itself. ACh is present in sensory nerves, although in small amounts (7). Furthermore, there is a release of ACh on stimulation of sensory nerves (and on stimulation only), as shown by Brecht and Corsten as early as 1942 (2). The amounts released are small and can be detected only by using the most sensitive but rather subtle method of ACh-determination: the frog lung test. The compound released showed all the properties of ACh (inactivation by cholinesterase, potentiation by eserine, etc.). It can be expected from those experiments that there must also be a mechanism for ACh-formation in sensory nerves.

In the first place, a crude calculation can be made in order to determine how much choline acetylase (ChAc) we might expect from the small amounts of ACh found. If the expected values are higher than can be detected, there is always the possibility that there are more ways of uptake of the acetyl radical for ACh-formation. A few months ago Berry and Stotz (1) presented evidence that ACh-synthesis is increased, and that ATP is not necessary when choline is replaced by phosphorylcholine. As they say, it would appear that with phosphorylcholine as a substrate, acetylation might occur without activation of an acetyl precursor. I am mentioning this preliminary communication only to indicate that there might be better ways of ACh-formation than we know of at the moment.

With respect to Dr. Hebb's results with Koelle's histochemical method, I think that I can leave this part of the paper to Dr. Koelle himself. Regarding, however, the controversies over this method and the different conclusions Dr. Koelle drew, using both his original and his improved method, I can quote him in saying that "the accuracy of the histochemical localization of ChE at an intracellular level is still questionable" (6); in my opinion it might be better to wait some years before this interesting method is used in the decision between the various theories of transmission or conduction.

Until then we depend upon the usual ways of determining ChE-activity. I was interested in Dr. Hebb's observation that the injection of DFP into the optic papilla did not affect light reflexes. Evidence of this kind leads immediately to the question of whether all the ChE was really inhibited. My own experiments with chronic DFP-poisoning in dogs led me to the conclusion that one must be very sure indeed before it can be stated that ChE-activity has been reduced to nearly zero. Special care must be taken of the following points. 1. If the ChE-activity after inhibition is small, it is necessary to investigate into the presence of free DFP in the tissues, otherwise this free DFP may inhibit ChE during the preparation of the enzyme extract. 2. When the CO_2-production in the manometric ChE-determinations is very small, it is often masked by a CO_2-uptake in the beginning of the experiments. A CO_2-production which is unob-

served or absent during the first 45 minutes may become very clear during the next two hours. 3. Even if no ChE-activity is found manometrically, activity can often be demonstrated by using the biological ChE-determination (assay for disappearance of amounts of ACh). 4. In this case, the choice of the initial substrate concentration can be of decisive importance for the results.

By paying attention to these points, it can be shown that tissues in which the ChE-activity seems to be reduced to zero still contain the active enzyme up to 10% of the original amount (8). These methodological problems were pointed out before (3), but I would like to stress them because they are often slighted. Prof. Gerard, for example, said a few years ago that his findings were criticized on methodological grounds, which arguments were not very impressive to him (4). In my opinion many experimental data can be explained by the fact that these points were not considered, one example being a paper by Heymans & Casier (5).

Some aspects of the small quantity of the ACh-ChE-ChAc-system in sensory nerves are not yet understood, but I doubt whether there is enough evidence up to now to assume a mechanism of conduction or transmission different from that of other nerves.

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