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THE ROLE OF GANGLIOSIDE IN THE MODE OF ACTION OF TETANUS TOXIN

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TETANUS toxin is a simple protein of molecular weight 67,000. Like the other bacterial neurotoxins, botulinum and dysentery, it is exceptionally toxic and may contain up to 70 million mouse LD_{50}/mg. It is therefore about half a million times as toxic as ouabaine, or 100,000 times as toxic as fluoracetate.

Eccles has shown that tetanus toxin suppresses the inhibitory postsynaptic potentials which can be recorded by intracellular microelectrodes from spinal motor neurones.[1] This provides direct neurophysiological evidence of a central action of the toxin which would explain the clinical picture of spastic paralysis. There are no gross or microscopic pathological signs in acute tetanus poisoning.[2] Recently we have made electron microscopic examinations of the anterior horns of spinal cords from rabbits dying from 500,000 mouse LD_{50} of tetanus toxin (75 per cent pure). So far no evidence has been found of abnormality in the boutons terminaux or the subsynaptic membranes.

At the biochemical level the only known reaction of tetanus toxin of possible relevance to its mode of action is its fixation by nervous tissue which appears to be due to the ganglioside in the tissue.[3–7] This fixation is avid, and both toxin- and ganglioside-specific. The reaction does not appear to involve any chemical change in the ganglioside. Ganglioside is a water-soluble lipid and because of its many hydrophilic and hydrophobic residues it has often been suggested as a component of cell membranes. We have shown that ganglioside forms water-insoluble complexes (mixed micelles?) with cerebroside and sphingomyelin. In aqueous solution ganglioside forms micelles of high particle weight and hence its combination with toxin can easily be demonstrated in the analytical ultracentrifuge, provided the concentration of toxin, is high (5 mg, or 250 million LD_{50}/ml). At this concentration of toxin, ganglioside may fix up to 20 times its weight of toxin. At low concentration of toxin, fixation can be measured by finding the least amount of nervous tissue or insoluble ganglioside complex which

will fix 10 LD_{50} of toxin as measured by assay in mice. At a toxin concentration of 40 LD_{50}/ml we found that the capacity of ganglioside to fix toxin was greatly increased when it was complexed with a particular proportion of cerebroside (which itself does not fix toxin). Thus a complex containing 25 per cent ganglioside fixes 200 times as much toxin as complexes containing 5 or 50 per cent ganglioside.[8]

Experiments at low concentrations of toxin might be more likely to yield useful information on the action of the toxin *in vivo*. Since ganglioside and cerebroside (and probably other substances) form complexes and since both are fairly abundant in the central nervous system they may well occur naturally in combination. In this case the "receptor sites" for tetanus toxin in the central nervous system may be a limited number of special "patches" where the ratio of ganglioside: cerebroside is optimal for fixation and these "patches" might in some way be involved in inhibitory transmission.

However, this avid and specific fixation of tetanus toxin by ganglioside may be fortuitous. The only evidence which may connect the fixation with the lethality of the toxin is that tetanus toxoid, which is the only other protein we have found to be fixed by ganglioside under our conditions, can prevent (at very high concentrations) both the fixation by brain *in vitro* and the lethal action *in vivo*.[9]

If fixation of tetanus toxin by nervous tissue is important in its action it is interesting to know to what morphological units the toxin is bound. We have done some preliminary experiments on the fixation of toxin by different subcellular fractions of brain.[8] Whittaker has devised methods by which relatively pure fractions containing nipped-off nerve endings[10] or isolated synaptic vesicles[11] may be obtained. The nerve endings fix about twice as much toxin per unit weight as the original brain homogenate; the synaptic vesicles fix less than the homogenate. If the fixation and the lethal action of tetanus toxin are connected, this might suggest that the toxin does not act on the transmitter-containing vesicles. An implication of this result, when one considers the possibility that ganglioside may be a component of excitable membranes, may be that tetanus toxin acts on the inhibitory pre- or postsynaptic membranes. It is difficult to envisage a mere physical occupation of certain membrane sites as a catalytic action and yet the high order of toxicity of the toxin suggests that we might expect to find some catalytic action for it.

Obvious examples of a possible catalytic action would be either that tetanus toxin is an enzyme which destroys the inhibitory transmitter, or that it blocks the formation of the transmitter. However, experience with botulinum toxin shows this is not the only way in which the release of transmitter from nerve endings may be suppressed.[12] This toxin is also a highly potent neurotoxin and is produced by a micro-organism closely related to *Cl. tetani*. It interferes with the release of acetylcholine from

nerve endings, and yet it does not inhibit the formation of acetylcholine, nor is it an acetylcholine esterase.

In this connection we have also shown that tetanus toxin does not affect acetylcholine, adrenaline, dopamine, histamine, 5-hydroxytryptamine, substance P or γ-aminobutyric acid as assayed on the isolated guinea-pig ileum.[8] In addition, since γ-aminobutyric acid may be involved in inhibition in the central nervous system,[13] we attempted to see whether an increase in its concentration in the central nervous system affected the toxicity of tetanus toxin. Daily injection of aminoxyacetic acid into mice, which among other things raises the γ-aminobutyric acid level,[14] did not affect the toxicity.[8]

The mode of action of tetanus toxin at the biochemical level is still obscure and the only reaction so far demonstrated *in vitro* remains its fixation by ganglioside. The relation, if any, of this reaction to the spasmolytic action of the toxin is an open question.

REFERENCES

1. Eccles, J. C. (1957) *The Physiology of Nerve Cells*, Oxford University Press, London.
2. Pillemer, L. and Wartman, W. B. (1947) *J. Immunol.* **55**, 277.
3. van Heyningen, W. E. (1959) *J. Gen. Microbiol.* **20**, 291.
4. van Heyningen, W. E. (1959) *J. Gen. Microbiol.* **20**, 301.
5. van Heyningen, W. E. (1959) *J. Gen. Microbiol.* **20**, 310.
6. van Heyningen, W. E. and Miller, P. A. (1961) *J. Gen. Microbiol.* **24**, 107.
7. Bernheimer, A. W. and van Heyningen, W. E. (1961) *J. Gen. Microbiol.* **24**, 121.
8. Mellanby, J. and van Heyningen, W. E., Unpublished.
9. Fulthorpe, J. (1956) *J. Hyg.* **54**, 315.
10. Grey, E. G. and Wittaker, V. P. (1962) *J. Anat. (Lond.)* **96**, 79.
11. Wittaker, V. P., Michaelson, I. A. and Kirkland, R.J.H. (1963) *Biochem. Pharmacol.* **12**, 300.
12. Wright, G. P. (1955) *Pharmacol. Rev.* **7**, 413.
13. Roberts, E. (1960) Editor, *Inhibition in the Nervous System and Gamma-aminobutyric Acid*. Pergamon Press, Oxford.
14. Roberts, E., Private communication.

DISCUSSION

Halbert, *U.S.A.*: Is the complex of tetanus toxin and ganglioside toxic, or is the toxicity of tetanus toxin in such a complex reduced appreciably?

Mellanby: The toxicity is reduced considerably but this may be due to the non-specific inactivation of the toxin by ganglioside before injection.

Halbert: If ganglioside is injected intraperitoneally will it protect animals against subsequent intraperitoneal injection of tetanus toxin?

I have in mind the potential application of excess ganglioside as a therapeutic agent during the early stages of tetanus intoxication in animals or man in the hope of reversing the presumed attachment of the toxin to the susceptible site.

MELLANBY: 5 mg ganglioside injected into a mouse intraperitoneally, intramuscularly or intravenously will protect the animal against 2 or 3 LD_{50} of tetanus toxin injected by the same or another route, on the same or the following day but not the previous day.

BOROFF, *U.S.A.*: Have you tried the effect of serotonin or other pharmacologically active compounds on tetanus intoxication *in vivo*?

MELLANBY: We have tried 5-hydroxytryptophane and this has no effect.

KRYZHANOVSKII, *U.S.S.R.*: What do you think about the connection between the absorption of tetanus toxin by ganglioside and its toxic action? Is the ganglioside only a physical receptor or does the reaction play a role in some toxic action?

MELLANBY: If the ganglioside forms a part of the postsynaptic membrane and if ganglioside is required for the excitation of this membrane, then maybe physical adsorption of toxin into specific ganglioside molecules could block transmission. I find it very difficult to think that this kind of physical occupation could explain the exceptional toxicity of tetanus toxin. Indeed there is no proof that the fixation of toxin by ganglioside is a necessary part of its lethal action, though I feel that such specific and avid reaction must have some biological significance.