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Mechanism of Action of Neurotoxins

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ABSTRACT

This paper is a summary of studies over the past few years that pertain to animal neurotoxins. These toxins are found throughout the animal kingdom. Homologies exist in the structures of these poisons within classes and point to conservation of active sites throughout evolution. In the case of the peptides, invariant amino acids may be involved in the active site, be essential for maintaining the shape and conformation of the molecule or serve as a fulcrum for folding of the peptide chain after synthesis. At the nuclear or DNA-level, a constant base sequence may regulate gene operation so that only a specific amino acid is coded.

Physiologically, and with ultrastructural and biochemical correlation, the predominant mode of action of neurotoxins relate to one or the other of the major activities of the excitable cell,—on conductile activity affecting Na^+ or K^+ permeabilities, on output or secretory activities affecting the release of neurotransmitter or on input generator activities affecting the receptor molecules for transmitter themselves. The future of these animal neurotoxins in neurobiological research is secure. The elucidation of molecular mechanisms, by which these various physiological activities of excitable tissue are expressed, will surely involve one or more of these fascinating, naturally-occurring compounds.

Introduction

A discussion of the effects of toxic agents on the nervous system could easily occupy a great deal of time. In terms of human exposure, as a health hazard, all are becoming acutely aware of the deleterious effects of inorganic and organic environmental pollutants. All age groups are at risk with industrial or accidental exposure in the high risk bracket. Mechanistically, a toxic agent, to be classified a neurotoxin, should express its adverse effects by interfering with some microphysiologic or biochemical reaction generally specific for the nervous system. Combination with neural receptor molecules, blocking of enzyme active sites, or formation of analogs or metabolites bearing some chemical characteristics of the native compound could all serve as mechanisms for neurotoxin effects. Additionally, neural function, *in vivo*, could be impaired in a secondary or tertiary fashion by the effects of toxins on other systems which may, in turn, impair its normal regulation.

Neurotoxins can be of mineral, plant or animal origin. In recent years, potent animal toxins have attracted steadily increasing attention. New techniques have developed for the isolation and purification of these naturally occurring neurotoxic compounds which have resulted in a rapid rise in our

understanding of their mechanism of action. These neurotoxic products, from a wide variety of animal sources, have become valuable tools for neurobiological research. For this reason, this paper will describe some of the potent neurotoxins and the state of our knowledge concerning their mechanism of action. Even with this restriction, this presentation is only a short summary and for further reading, proceedings of the three recent major symposia and several review articles are strongly recommended.[25,30,50]

Neurotoxins can be found throughout the animal kingdom. The cause of paralytic shellfish poisoning has been shown to be caused by release of toxins by several protozoan species (expecially *Gonyaulax catenella*) which accumulate in the siphons of the shellfish feeding on these dinoflagellates.[49] In Arthropoda, the smallest neurotoxic peptide, apamin, has been isolated from bee venom. A number of neurotoxins have been isolated from various scorpion venoms[15,42,46] and black widow spiders.[15,37,44]

In the vertebrates, neurotoxic substances have been shown in fishes since antiquity. Tetrodotoxin has been isolated in crystalline form from the puffer fish and its structure elucidated.[53] Amphibians, such as frogs, toads and salamanders produce neurotoxins found largely in skin glands. Many of these compounds are highly toxic steroidal alkaloids and are used to dip poison arrows by Indians of the Colombian rain forests. Batrachotoxin (BTX) has been one such major toxic compound isolated.[18] Recently, another toxic compound, histrionicotoxin (HTX), has been shown to have apparently specific neural actions.[4,5,17]

In the reptiles, however, where sociological interest has been present since primeval times, the most widely known neurotoxins are those isolated from the venoms of poisonous snakes. In recent years, toxins found in snakes of the families *Elapidae* (cobras, mambas, kraits, etc.) and *Hydrophidae* (sea-snakes) have been widely used in research on cholinergic transmission in the nervous system.[34]

Mechanism of Action

To discuss the probable mechanism of action of a few select animal neurotoxins, it would be profitable to separate sites of action both anatomically and physiologically. This is somewhat arbitrary but tends to distinguish the various functions of excitable cells as outlined by Grundfest.[26] These functions, if not delineated geographically, can be separated effectively by differing physiologic responses and propperties. In general, the neuron, as the unit of activity of the nervous system, consists of input or generator activity, conductile activity, and output or secretory activity. The neurotoxins under consideration here have been shown to effect specific sites relating to these activities. They are, therefore, in their most predominant mechanism of action: postsynaptic, axonal or presynaptic neurotoxins.

Axonal Neurotoxins

Tetrodotoxin (TTX), an amino-perhydroquinazoline compound found in tissues of fishes in the order *Tetraodontiformes* (puffer fish, fugu, etc.) and in the California newt, inhibits the action potential of nerve and muscle membranes.[31,40] It has been shown that tetrodotoxin blocks the transient inward flow of sodium ions (blocks sodium conductance) which prevents depolarization. Saxitoxin (STX) having the same potency and action as TTX, is found in clams and mussels only when the shellfish have been feeding on certain dinoflagellates. It is responsible for the syndrome of paralytic shellfish poisoning.[47,48,49] Since the conductile mechanisms and properties of axonal and muscle membranes are similar, it is not surprising that these neurotoxins have similar effects on blocking both neuronal and muscle action potentials. Recently,

using ^{3}H-labelled TTX partial characterization of a binding-component in membrane particles of unmyelinated garfish olfactory nerve has been demonstrated.[7,28] This component is presumed to be a protein embedded in a phospholipid environment in the membrane. From previous studies, it had been shown that neither active Na^+ transport nor NaK ATPase activity[31,38] is affected by TTX.

Batrachotoxin (BTX), a steroidal alkaloid obtained from the frog, *Phyllobates aurotaenia,* causes depolarization of electrically-excitable membranes.[3,7] BTX appears to exert its action on nerve and muscle by irreversibly increasing permeability to Na^+. Although both TTX and BTX influence permeability to Na^+, both probably act on different receptor substances. It has been shown that denervated muscles become insensitive to TTX but remain sensitive to the action of BTX. It is possible, however, that denervation induces some structural or conformational changes in Na^+ permeability molecules which would dissociate sensitivity to these toxins.

Presynaptic Neurotoxins

The most potent presynaptic neurotoxin is not of animal origin but is produced by the growth of a bacterium, *Clostriduim botulinum.* Mention is made here because in its purified form, botulinus toxin is the most toxic biological substance known.[51] Animal neurotoxins with a presynaptic mode of action can be found in scorpion venoms and in the black widow spider venom as well as from several snake venoms.

In Mexico, from 1940 to 1949 and 1957 to 1958, 10 times as many people were killed by scorpion stings than from snake bites.[39] Some 10 or 11 basic peptides have been isolated from scorpion venom.[42] These toxins have been shown to cause depolarization of nerve and muscle membranes,[46] probably owing to increased sodium permeability. In this respect they are similar to BTX since they are antagonized by either reducing the external sodium concentration, increasing calcium concentration, or by TTX.[1]

Recently, however, at peripheral adrenergic synapses, it has been suggested that a toxin from *Leiurus quinquestriatus* causes transmitter release[43] indicating a presynaptic terminal mode of action. Earlier reports suggested this presynaptic mechanism at cholinergic neuromuscular junctions.[19] This type of mechanism has been determined for black widow spider venon (BWSV) by several laboratories.[15,37,44] The specific toxin has not been purified and analyzed as yet. Its mechanism of action appears to be one of interacting, somehow, with nerve terminal membrane to affect "avalanches" of transmitter release until all stores are exhausted. It is independent of Ca^{++} and terminal depolarization. Correlation of ultrastructure and physiological studies show depletion of presynaptic vesicles when no further release occurs.

Venoms of several snakes contain toxins which appear specific for presynaptic mechanisms. One such neurotoxin, isolated from the venom of the banded krait, *Bungarus multicinctus,* has been named Beta-bungarotoxin (B-BuTX). This ca. 25,000 M.W. basic peptide appears to act exclusively on the presynaptic side by inhibiting the release of acetylcholine (ACh).[11,12] An initial burst of ACh release occurs and correlates with depletion of synaptic vesicles.[14] Recently, inhibition of Ca^{++} accumulation into rat brain mitochondria by B-BuTX has been demonstrated.[54] Since Ca^{++} influx is necessary for release of transmitter these authors conclude that the presynaptic action B-BuTX might be through alteration of mitochondrial Ca^{++} metabolism. Crotoxin, derived from the venom of the South American rattlesnake, is one of the first neurotoxins isolated from snake venom.[52] It is a complex of a non-toxic acidic protein and a basic phospholipase. It is also somewhat complex in its actions, having both pre- and post-junctional effects. It is of interest that presynaptic mechanisms predominate in

amphibian junctions, while in mammalian systems the mechanisms are primarily postsynaptic.[10]

Recently, another snake toxin isolated from the Australian tiger snake (*Notechis scutatus*) has been shown to block transmitter release.[27] However, complete depletion of vesicles was not found despite the physiological findings of an increase in miniature end-plate potential (MEPP) frequency and then followed by a decline and ultimate cessation of MEPPs. These presynaptic toxins isolated from scorpion venom, BWSV, and the several snake venoms all appear to support the vesicle hypothesis[29] of synaptic transmission. One must be cautious, however, that although correlation of structure and function is of utmost importance in elucidating basic mechanisms, they may not be causally related.

Postsynaptic Neurotoxins

In the search for receptor molecules in neurobiology the use of postsynaptic-acting snake neurotoxins has brought considerable progress related to isolation and purification of ACh receptors. As in the scorpion toxins, significant homologies exist in the structures of these small basic peptides isolated from snakes of the families *Elapidae* and *Hydrophidae*.[13,34] These toxins are curarimimetic but are more than 30 times as potent as curare. In spite of this, they are significantly less toxic than the presynaptic acting snake neurotoxins.[33] They are small basic peptides tightly cross-linked by disulfide bridges. The high degree of disulfide cross-linking accounts for stability at high temperatures or on exposure to 8 M urea.[55] However, they are rapidly inactivated by strong alkali and by reduction of the disulfide bonds. Within this class there are two groups: smaller (Ca 7000 mol. wts.) containing 60 to 62 amino acids and four disulfide bridges, and larger (Ca 8000) with 71 to 74 amino acids and five disulfides.[34] Prototypes of the small type are the sea-snake neurotoxins and the principal neurotoxins in the majority of cobra species. Of the larger group, the best known are alpha-bungarotoxin (alpha-BuTX) and the cobrotoxin of *N. naja naja* and *N. naja siamensis*. Chemically, the larger toxins, in addition to containing more amino acids and the important fifth disulfide bridge, contain more hydrophobic amino acids such as alanine, phenylalanine and valine.[34]

The larger and smaller curarimimetic neurotoxins differ in other respects. The affinity for the ACh receptor is greater for the larger toxins than for the smaller. Alpha-BuTX binding has been referred to as "essentially irreversible".[34] Antisera directed against one group is specific for the group but doesn't neutralize toxin from the other size group.[9] Smaller postsynaptic snake neurotoxins are also more susceptible to chemical degradation than are the larger ones.[32]

Utilizing labelled alpha-BuTX or larger cobra toxins, receptor molecules have been isolated, purified and solubilized from the electric organs of the eel (*Electrophorus electricus*) and the fish (Torpedo Spp). In addition, the binding of ^{125}I-BuTX to particulate membranes and solubilized skeletal muscle membrane molecules identified as nicotinic cholinergic receptors by certain criteria, has been demonstrated by several laboratories.[6,8,23,24] Using a toxin-binding assay for identifying the receptor, anti-receptor antibodies have been produced which resulted in a paralytic syndrome suggestive of neuromuscular transmission blockade.[45] Recently, using ^{125}I-BuTX, separation of membranes enriched for acetylcholinesterase (AChE) from the receptor was demonstrated in electric tissue[21] and in skeletal muscle membranes.[24] The human disease myasthenia gravis (MG) has, at times, been thought to be due to either a presynaptic or postsynaptic defect. Supportive but not conclusive evidence for a postsynaptic mechanism has recently been reported. Muscle from biopsies of MG patients appeared to bind less ^{125}I-BuTX

than normals and disease controls.[22] In addition, a serum globulin from MG patients has been reported to block ^{125}I-BuTX finding to solubilized, denervated (extrajunctional) receptors but not to normal junctional receptors of rat membranes.[6] It is, at present, not clear as to the significance of these findings in understanding the pathophysiology of the disorder.

References

1. Adam, K. R., Schmidt, H., Stampfli, R., and Weiss, C.: The effect of scorpion venom on single myelinated nerve fibers of the frog. Brit. J. Pharmacol. *26*:666–677, 1966.
2. Albuquerque, E. X., Warnick, J. E., and Sanone, F. M.: The pharmacology of batrachotoxin. II. Effect on electrical properties of the mammalian nerve and skeletal muscle membranes. J. Pharmacol. Exp. Ther. *176*:511–528, 1971.
3. Albuquerque, E. X.: The mode of action of batrachotoxin. Fed. Proc. *31*:1133–1138, 1972.
4. Albuquerque, E. X., Kuba, K., and Daly, J.: Effect of histrionicotoxin on the ionic conductance modulator of the cholinergic receptor: A quantitative analysis of the end-plate current. J. Pharmacol. Exp. Ther. *189*:513–524, 1974.
5. Albuquerque, E. X., Barnard, E. A., Chiu, T. H., Lapa, A. J., Dolly, J. O., Jansson, S. E., Daly, J., and Witkop, B.: Acetylcholine receptor and ion conductance modulator sites at the murine neuromuscular junction: Evidence from specific toxin reactions. Proc. Nat. Acad. Sci. *70*:949–953, 1973.
6. Almon, R. R., Andrew, C. G., and Appel, S. H.: Serum globulin in myasthenia gravis: Inhibition of alpha-bungarotoxin binding to acetylcholine receptors. Science *186*:55–57, 1974.
7. Benzer, T. T. and Raftery, M. A.: Solubilization and partial characterization of the tetrodotoxin binding component from nerve axons. Biochem. Biophys. Res. Comm. *51*:939–944, 1973.
8. Berg, D. K., Kelly, R. B., Sargent, P. B., Williamson, P., and Hall, Z.: Binding of alpha-bungarotoxin to acetylcholine receptors in mammalian muscle. Proc. Nat. Acad. Sci. *69*:147, 1972.
9. Boquet, P., Polleux, G., Dumarey, C., Izaro, V., and Ronsseray, A. M.: An attempt to classify the toxic proteins of Elapidae and Hydrophidae venoms. Toxicon *11*:333–340, 1973.
10. Brazil, O. V. and Excell, B. J.: Action of crotoxin and crotactin from the venom of *Crotalus duris sus terrificus* (South American rattlesnake) or the frog neuromuscular junction. J. Physiol. *212*:341, 1970.
11. Chang, C. C., Cacn, T. F., and Lee, C. Y.: Studies on the presynaptic effect of B-bungarotoxin on neuromuscular transmission. J. Pharmacol. Exp. Ther. *184*:339–345, 1973.
12. Chang, C. C. and Huang, M. C.: Comparison of the presynaptic actions of Botulinum toxin and B-bungarotoxin on neuromuscular transmission. Naunyn-Schmiedeberg's Arch. Pharmacol. *282*:129–142, 1974.
13. Chang, C. C. and Lee, C. Y.: Isolation of neurotoxins from the venom of Bungarus multicicntus and their modes of neuromuscular blocking action. Arch. Int. Pharmacodyn. *144*:241–257, 1963.
14. Chen, J. L. and Lee, C. Y.: Ultrastructural changes in the motor nerve terminals caused by B-bungarotoxin. Vichows Arch. Abt. B. Zellpath. *6*:318–325, 1970.
15. Cheymol, J., Bourillet, F., and Roch-Arveiller, M.: Venins et toxines de scorpions effects neuromusculaires. Actual. Pharmac. *25*:241–258, 1972.
16. D'Ajello, V., Magni, F., and Bettini, S.: The effect of the venom of the black widow spider *Latro dectus mactans tredecimguttatus* on the giant neurones of *Peridlaneta Americana*. Toxicon *9*:103–110, 1969.
17. Daly, J. W., Karle, I., Myers, C. W., Tokuyama, T., Waters, J. A., and Witkop, B.: Histrionicotoxins: Roentgen-ray analysis of the novel allenic and acetylenic spiroalkaloids isolated from a Colombian frog. *Dendrobates hystrionicus*. Proc. Nat. Acad. Sci., *68*:1870–1875, 1971.
18. Daly, J. and Witkop, B.: Batrachotoxin, an extremely active cardio- and neurotoxin from the Colombian arrow poison frog *Phyllobates Aurotaenia*. Clin. Toxicol. *4*:331–342, 1971.
19. Del pozo, E. C., Salas, M., and Pacueco, P.: Effects of scorpion venom at neuromuscular junction. Mems. Inst. Butantan. *33*:3, 1961.
20. Dettbarn, W. D.: Mechanism of Action of Tetrodotoxin (TTY) and Saxitoxion (STX). Neuropoisons: Their Pathophysiological Actions. Simpson, L. L., ed. New York, Plenum Press, vol. 1, p. 169, 1971.
21. Duguid, J. R., and Raftery, M. A.: Fractionation and partial characterization of membrane particles from *Torpedo californica* electroplax. Biochemistry *12*:3593–3597, 1973.
22. Fambrough, D. M., Drachman, D. B., and Satyamurti, S.: Neuromuscular junction in myasthenia gravis: Decreased acetylcholine receptors. Science *182*:293–295, 1973.
23. Fambrough, D. M., Hartzell, H. C., Powell, J. A., Rash, J. E., and Joseph, N.: On the differentiation of the surface membrane of a postsynaptic cell. The Neuronal Interaction. Bennett, M. V. L., ed. New York, Raven Press, pp. 285–313, 1974.
24. Festoff, B. W. and Engel, W. K.: *In vitro* analysis of the general properties and junctional receptor characteristics of skeletal muscle membranes. Isolation, purification and partial characterization of sarcolemmal fragments. Proc. Nat. Acad. Sci. *71*:2435–2439, 1974.
25. First International Symposium on Animal Toxins, Atlantic City, NJ, 1966. Animals Toxins. Russell, F. E. and Saunders, P. R., eds., New York, Pergamon Press, 1967.
26. Grundfest, H.: On the how and why of synapses. Synaptic Transmission and Neuronal Interaction.

Bennet, M. V. L., ed. New York, Raven Press, pp. 1–21, 1974.
27. Harris, J. B., Karlsson, E., and Thesleff, S.: Effects of an isolated toxin from Australian Tiger Snake (*Notechis scutatus scutatus*) venon at the mammalian neuromuscular junction. Brit. J. Pharmacol. *47:*141–146, 1973.
28. Henderson, R. and Wang, J. H.: Solubilization of a specific tetrodotoxin-binding component from garfish olfactory nerve membrane. Biochemistry *11:*4565–4569, 1972.
29. Hubbard, J. I.: Microphysiology of vertebrate neuromuscular transmission. Physiol. Rev. *53:*674–723, 1973.
30. International Symposium on Animal Venoms, Sao Paulo, Brazil, 1966. Memorial Institute of Butantan *33:*1–1022, 1966.
31. Kao, C. Y.: Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomena. Pharmac. Rev. *18:*997–1048, 1966.
32. Karlsson, E., Eaker, D., Fryklund, L., and Kadin, S.: Chromatographic separation of *Enhydrina schistosa* (common sea-snake) venom and characterization of two principal neurotoxins. Biochemistry *11:*4628–4633, 1972.
33. Karlsson, E.: Chemistry of some potent animal toxins. Experientia *29:*1319–1327, 1973.
34. Lee, C. Y.: Chemistry and pharmacology of polypeptide toxins in snake venoms. Ann. Rev. Pharmacol. *12:*265–286, 1972.
35. Lee, C. Y. and Chang, C. C.: Modes of actions of purified toxins from elapid venoms on neuromuscular transmission. Mem. Inst. Butantan. *33:*555–572, 1966.
36. Lee, C. Y., Chang, S. L., Kau, S. T., and Luh, S. H.: Chromatographic separation of the venom of *Bungarus multicinctus* and characterization of its components. J. Chromatogr. *72:*71–82, 1972.
37. Longenecker, H. E., Jr., Hurlburt, W. P., Mauro, A., and Clark, A. W.: Effects of black widow spider venom on the frog neuromuscular junction. Nature *225:*701–705, 1970.
38. Marumo, F., Yamada, T., Asano, Y., Sasaoka, T., Yoshida, A., and Endou, H.: Role of the inhibitory effect of tetrodotoxin on the active sodium transport of the toad bladder. Pflugers Arch. *303:*49–54, 1968.
39. Mazzotti, L. and Bravo-Becherelle, M. A.: Scorpionism in the Mexican Republic in Venomous and Poisonous Animals and Plants in the Pacific Region. Keegan, H. L. and MacFarlane, W. V., eds. New York, Pergamon Press, p. 119, 1963.
40. McCrone, J. D. and Hatla, R. J.: Animal Toxins. Russel, F. E., and Saunders, P. R., ed., New York, Pergamon Press, p. 29, 1967.
41. Miledi, R. and Potter, L. T.: Acetylcholine receptors in muscle fibers. Nature *229:*554–557, 1971.
42. Miranda, F., Kupeyan, C., Rochat, H., Rochat, C., and Lissitzsky, S.: Purification of animal neurotoxins: Isolation and characterization of eleven neurotoxins from the venom of scorpions. Eurp. J. Biochem. *16:*514–523, 1970.
43. Moss, J., Thoa, N. B., and Kopin, I. J.: On the mechanism of scorpion toxin-induced release of norepinephrine from peripheral adrenergic neurons. J. Pharmacol. Exp. Ther. *190:*39–48, 1974.
44. Okamoto, M., Longenecker, H., Riker, W. F., and Clark, A. W.: *Destruction of Mammalian motor* nerve terminals by black widow spider venom. Science *172:*733–736, 1971.
45. Patrick, J. and Lindstrom, J.: Autoimmune response to acetylcholine receptor. Science *180:*871–872, 1973.
46. Rochat, H., Rochat, C., Kupeyan, C., Miranda, F., Lissitzsky, S., and Edman, P.: Scorpion neurotoxins: A family of homolous proteins. FEBS Let. *10:*349–351, 1970.
47. Schantz, E. J., Mold, J. D., Stanger, D. W., Shauel, J., Riel, F. J., Bowden, J. P., Lynch, J. M., Wyler, R. S., Riegel, B., and Sommer, H.: Paralytic shellfish poison. IV. A procedure for isolation and purification of the poison from toxic clam and mussel tissues. J. Amer. Chem. Soc. *79:*5230–5235, 1957.
48. Schantz, E. J., Lynch, J. M., Vayvada, G., Matsumoto, K., and Rapoport, H.: The purification and characterization of the poison produced by *Gonyaulax catenella* in Axenic culture. Biochemistry *5:*1191–1195, 1966.
49. Schantz, E. G.: Paralytic shellfish poisoning and saxitoxin. Neuropoisons: Their Pathophysiological Actions. Simpson, L. L., ed., New York, Plenum Press, vol. 1, p. 159, 1971.
50. Second International Symposium on Animal and Plant Toxins, Tel Aviv, Israel, 1970. Toxins of Animal and Plant Origin. De Vries, A. and Kochva, E., eds. London, Gordon and Breach, 1966.
51. Simpson, L. I.: The neuroparalytic and hemagglutinating activities of botulinum toxin. Neuropoisons: Their Pathophysiological Actions. Simpson, L. L., ed. New York, Plenum Press, vol. 1, pp. 303–323, 1971.
52. Slotta, K. and Fraenkel-Conrat, H. Schangensifle, III. Mitteil: Reinigung und Krystallisation des Klapper-schlangen-Giftes. Chem. Ber. *71:*1076–1081, 1938.
53. Tsuda, K., Tachikawa, R., Sakai, K., Tamura, C., Amakasy, O., Kawamura, M., and Ikuma, S.: On the structure of tetrodotoxin. Chem. Pharmac. Bull. *12:*642–645, 1964.
54. Wagner, G. M., Mart, P. E., and Kelly, R. B.: B-Bungarotoxin inhibition of calcium accumulation by rat brain mitochondria. Biochem. Biophys. Res. Comm. *58:*475–481, 1974.
55. Yang, C. C.: The disulfide bonds of cobrotoxin and their relationship to lethality. Biochem. Biophys. Acta *133:*346–355, 1967.