A Deadly Poison Becomes a Useful Tool

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JAPANESE ROULETTE

Japanese have long loved to eat "very delicious" puffer fish (also known as "blowfish" or "globefish") which they call "fugu" although it is known to contain a deadly poison. The many deaths attributed to the eating of puffer fish have caused the poison to be of special interest to Japanese scientists. Recent laws have restricted the serving of fugu fish to licensed restaurants where the chefs have been trained to remove the organs where the poison is most concentrated. These organs (the poison is most concentrated in the ovaries) are collected by a chemical and drug concern and the poison extracted. It is called tetrodotoxin from the family name Tetraodontidae.

ELECTROPHYSIOLOGY OF TETRODOTOXIN

Although studies of the effects of this poison have been done for some years on whole animals, nerve trunks and muscles, it has been used in single cell electrophysiological studies only quite recently.

Narahashi et al. reported in 1960 that tetrodotoxin (which hereafter will be called TTX) blocked excitability in skeletal muscle membrane without changing its slow electrical rectifying properties. About the same time Furukawa et al. (1959) found that the response of the muscle end plate region retained its sensitivity to acetylcholine although the excitation process itself was blocked.

Dr. Narahashi joined me at Duke in 1962 and we tested his hypothesis that TTX selectively blocked the sodium conductance increase associated with excitable membranes. We used the voltage clamp technique (which separates sodium and potassium membrane currents into two distinct time courses) on single giant axons from lobsters. It was found that the poison did indeed block the sodium flow very selectively and at almost incredibly low concentrations (10^{-8} to 10^{-7} molar).

About this time another toxin, tarichatoxin, thought to be somewhat different but almost equally powerful in blocking nerve impulses (Kao & Fuhrman, 1963) was isolated from eggs of the California newt *Taricha forosa* (Brown & Mosher, 1963).

Dr. Takata and I with Kao and Fuhrman tested tarichatoxin by the same technique on lobster axons and found its effect to be the same as that of TTX. Shortly thereafter it was shown by a number of chemical and physical techniques that the two toxins were in fact identical (Buchwald et al., 1964). An excellent review of the symptoms, physiology and pharmacology of TTX poisoning up to 1964 was written by Mosher et al. (1964).

RECENT RESULTS

Similar selective blockage of the voltage-sensitive sodium conductance systems has been observed in squid axon, single nodes of frog nerves and in the eel electroplax (Nakamura et al., 1964). It does not appear to affect smooth muscle or barnacle muscle. The latter is thought to be made excitable by an increase in conductance to calcium ions (Hagiwara & Naka, 1964). It appears to affect cardiac muscle only in a very limited way (Hagiwara & Nakajima, 1965).

Studies with internally perfused squid axons have shown that the inside may be perfused with a relatively high concentration of TTX without effect on the axon's excitability (Narahashi et al., 1966). This would seem to clearly localize the site of excitation on the outside surface of the membrane rather than on the inside as had often been supposed.

TTX (OR DERIVATIVES) AS AN ANESTHETIC

Voltage Clamp studies on a number of axons have shown that the mechanism of the excitation block by TTX is distinctly different from that caused by procaine.

Concentration Ratio for Blocking

Procaine reduces the ionic conductance of squid and lobster axon membranes at a concentration of about 3 to 4 mM (Taylor, 1959; Blaustein and Goldman, 1965) and blockage is usually sure with a concentration of 10 mM. In contrast, a concentration of only 90 mM of

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TTX blocks within five minutes. The toxin is therefore more effective by a factor of 10^5 in terms of the concentration required.

Selectivity

The toxins have been found to block only the sodium entry, leaving the potassium current unaffected (the present results; Narahashi et al., 1965; Nakamura et al., 1964; Moore, 1965). On the other hand, procaine affects the magnitude of both ionic conductances (Taylor, 1959; Blaustein and Goldman, 1965).

Time Course of the Conductance Changes

Our results show that the toxins do not alter the kinetics of the sodium or potassium current increase. Taylor (1959), Blaustein and Goldman (1965), and unpublished experiments in our laboratory have shown that procaine causes a distinct increase in the time for the sodium current to reach its peak. There is also a very marked slowing in rise of the late potassium current (Taylor, 1959; unpublished experiments in our laboratory).

Location of Action

Procaine appears to be effective in blocking nerve excitation when internally perfused in the squid axon at a concentration which blocks externally (1 to 10 mM, Narahashi, et al., 1966). In contrast, TTX has been found to be ineffective when internally perfused at a high concentration (1,000 mM) for long times (30 minutes). Preliminary evidence for TTX being ineffective on the inside of the membrane was shown by Moore (1965).

Interaction with Calcium

Blaustein and Goldman (1965) report that procaine appears to act at the same site that calcium does. Both cause shifts of the conductance curve along the voltage axis and alter the time course of the conductance changes. Although our experiments with tarichatoxin were not designed to study this point, it is clear that high calcium gives some protection against the toxin and definitely enhances the ability of the nerve to recover from a strong toxin depression of conductance.

The very high potency of TTX and its highly selective block of nerve and skeletal muscle fibers has already shed a great deal of light on their excitation properties and should lead to the ability to specify an entirely new type of anesthetic agent even if the toxin itself does not also turn out to be useful agent in this respect.

REFERENCES

BLAUSTEIN, M. AND D. E. GOLDMAN. Competitive action of calcium and procaine on lobster giant axons. *Fed. Proc.* 24: 584, 1965 (abstr).

BROWN, M. S. AND H. S. MOSHER. Tarichatoxin: isolation and purification. *Science* 140: 295–296, 1963.

BUCHWALD, H. D., L. DURHAM, H. G. FISCHER, R. HARADA, H. S. MOSHER, C. Y. KAO AND F. A. FUHRMAN. Identity of tarichatoxin and tetrodotoxin. *Science* 143: 474–475, 1964.

FURUKAWA, T., T. SASAOKA AND Y. HOSOYA. Effects of tetrodotoxin on the neuromuscular junction. *Jap. J. Physiol.* 9: 143–152, 1959.

HAGIWARA, S. AND K. NAKA. The initiation of spike potential in barnacle muscle fibers under low intracellular Ca^{++}. *J. Gen. Physiol.* 48: 141–162, 1964.

HAGIWARA, S. AND S. NAKAJIMA. Tetrodotoxin and manganese ion: effects on action potential of the frog heart. *Science* 149: 1254–1255, 1965.

KAO, C. Y. AND F. A. FUHRMAN. Pharmacological studies on tarichatoxin, a potent neurotoxin. *J. Pharmacol. Exp. Therap.* 140: 31–40, 1963.

MOORE, J. W. Voltage clamp studies on internally perfused axons. *J. Gen. Physiol.* 48: 11–17, 1965.

MOSHER, H. S., F. A. FUHRMAN, H. D. BUCHWALD AND H. G. FISCHER. Tarichatoxin-tetrodotoxin: a potent neurotoxin. *Science* 144: 1100–1110, 1964.

NAKAMURA, Y., S. NAKAJIMA AND H. GRUNDFEST. Selective block of Na-activation in voltage-clamped squid giant axon and eel electroplaque by tetrodotoxin. *Biol. Bull.* 127: 382, 1964.

NARAHASHI, T., N. ANDERSON AND J. W. MOORE. Effect of tetrodotoxin and procaine on the excitability of internally perfused squid axons. *Biophys. Soc. Abstracts,* 1966.

NARAHASHI, T., T. DEGUCHI, N. URAKAWA AND Y. OHKUBO. Stabilization and rectification of muscle fiber membrane by tetrodotoxin. *Am. J. Physiol.* 198: 934–938, 1960.

NARAHASHI, T., J. W. MOORE AND W. R. SCOTT. Tetrodotoxin blockage of sodium conductance increase in lobster giant axons. *J. Gen. Physiol.* 47: 965–974, 1965.

TAYLOR, R. E. Effect of procaine on electrical properties of squid axon membrane. *Am. J. Physiol.* 196: 1071–1078, 1959.