Short communications

Comparison of the actions of saxitoxin and tetrodotoxin on the motor end-plate of frog muscle

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End-plate potentials (e.p.p.) and responses to ionophoretic application of acetylcholine (ACh potentials) have been recorded in frog muscle. Saxitoxin (STX) and tetrodotoxin (TTX) abolished the e.p.p. at concentrations of 10^{-8} g/ml. In almost all cases there was no effect on the ACh potentials even when the concentrations were raised, STX to $1{\cdot}2\times10^{-6}$ g/ml ($3{\cdot}2\times10^{-6}$M), or TTX to 5×10^{-6} g/ml ($1{\cdot}5\times10^{-5}$M). STX, 10^{-6} g/ml ($2{\cdot}7\times10^{-6}$M) often caused a slow rise in the frequency of miniature e.p.p., without significant effect on amplitude.

Furukawa, Sasaoka & Hosoya (1959) showed that acetylcholine depolarized the motor end-plate in a muscle paralysed by tetrodotoxin. Tetrodotoxin renders nerve and skeletal muscle inexcitable but is without effect on chemical transmission at the neuromuscular junction (Katz & Miledi, 1967).

Saxitoxin has actions on nerve and muscle membrane that are almost identical with those of tetrodotoxin (Kao, 1966; Hille, 1970). It washes out more rapidly and completely than tetrodotoxin (Hille, 1968), is more stable, and is at least as potent on a molar basis. However, in spite of the advantages, it has not been widely used in studies of chemical transmission. It would be unsuitable if it had some effect on chemical transmission. Nishiyama (1967) reported that the sensitivity of the frog motor end-plate to acetylcholine was not reduced by saxitoxin (10^{-8} g/ml), but Kao & Nishiyama (1965) had reported a diminution in the size of miniature end-plate potentials (m.e.p.p.) after prolonged exposure to saxitoxin (10^{-6} g/ml).

Methods.—Sartorius or extensor longus digiti IV nerve-muscle preparations were dissected from pithed frogs (*Rana temporaria*). The nerve-muscle preparation was irrigated with oxygenated Ringers solution at 2·5 ml/minute. The composition was (mM) NaCl, 110; KCl, 2·5; $CaCl_2$, 1·8; glucose, 5·55, buffered to pH 7·3–7·4 with Tris (hydroxymethyl) methylamine-HCl, 5 or 10 mM. Usually the preparation was later paralysed by substituting $MgCl_2$ for an osmotically equivalent amount of NaCl. Saxitoxin, purified from *Saxidomus giganteus, Mytilus californianus* or *Gonyaulax catenella*, concentrations below 5×10^{-8} g/ml were made from a solution of 10 μg/ml ($2{\cdot}7\times10^{-5}$M) in 0·01 N HCl, diluted with Ringers solution. The small amount of acid did not alter the pH perceptibly. Higher concentrations were made from 100 μg/ml in 0·1 N HCl; this was mixed with an equal volume of 0·1 N NaOH and 2 volumes of double strength Ringers solution to give 25 μg/ml in Ringers solution; this was usually diluted with oxygenated Ringers solution to give a final concentration in the range $2\text{-}12\times10^{-7}$ g/ml. The pH was then checked to ensure that it was 7·3–7·4.

Tetrodotoxin (Sankyo Co.) was kept as a solution containing 10 μg/ml ($3{\cdot}13\times10^{-5}$M) in citric acid 50 μg/ml ($2{\cdot}6\times10^{-4}$M). It was diluted with oxygenated Ringers solution; the small amount of citric acid did not alter pH perceptibly and would not be expected to affect $[Ca^{2+}]$ appreciably.

Intracellular potentials were recorded through KCl-filled micropipettes. Membrane potentials and slow depolarizations were recorded with a potentiometric chart recorder and with a digital voltmeter which drove a printer. End-plate potentials (e.p.p.) were displayed on an oscilloscope and recorded photographically. The amplitudes of all responses were measured from photographs. The m.e.p.p. frequency was sometimes determined from photographic records, but in the later experiments the m.e.p.p. were counted by a frequency meter and recorded by the printer.

Acetylcholine (ACh) was applied ionophoretically from micropipettes filled with 4 M ACh (acetylcholine bromide, Sigma) positioned near to the end-plate region. Details of the technique resembled those described by del Castillo & Katz (1955).

Results.—The experiments in which ACh was applied by ionophoresis were carried out on sartorius nerve-muscle preparations. After establishing control values for resting membrane potential, e.p.p. and ACh potentials, freshly diluted toxin was run through the muscle chamber. The e.p.p.'s were re-

duced in amplitude by low concentrations of saxitoxin, disappearing within 2-3 min of exposure to 10^{-8} g/ml. This had no effect on the ACh potentials, and in almost every experiment the ACh potentials remained constant even when the saxitoxin concentration was increased. Concentrations up to $1{\cdot}2 \times 10^{-6}$ g/ml were tested for periods up to 6 minutes. In only one out of 11 experiments there appeared to be a 25-30% reduction in the amplitude of the ACh potential coinciding with a 3·6 min exposure to saxitoxin 10^{-6} g/ml, following a 16 min exposure to saxitoxin 10^{-8} g/ml.

In four experiments tetrodotoxin was added in concentrations up to 5×10^{-6} g/ml without significant effect on the ACh potentials. This confirms the finding of other workers.

The behaviour of m.e.p.p. responses in the presence of saxitoxin was studied mainly with the toe muscle preparation, although a few observations were also made on the smaller m.e.p.p. of the sartorius. In all cases, low concentrations of saxitoxin abolished the e.p.p. but the m.e.p.p. were not reduced in amplitude even after more than 40 min in concentrations up to 10^{-6} g/ml. In Fig. 1 the mean amplitude is not significantly different in the presence of saxitoxin (5×10^{-7} g/ml), in spite of a slow deterioration of the resting membrane potential.

However, the m.e.p.p. frequency often rose dramatically after a few minutes in high concentrations of saxitoxin. This is shown in Fig. 1, where m.e.p.p./100 s is plotted. After an initial fall, the frequency rose to about twice the control level, and remained high for 20-30 min after washing out the saxitoxin. Tubocurarine suppressed the m.e.p.p. when added as a control at the end of the experiment.

Discussion.—Tetrodotoxin is now widely used by workers investigating synaptic and neuromuscular transmission, free from the electrical changes associated with propagated action potentials. There is good evidence that tetrodotoxin has no influence

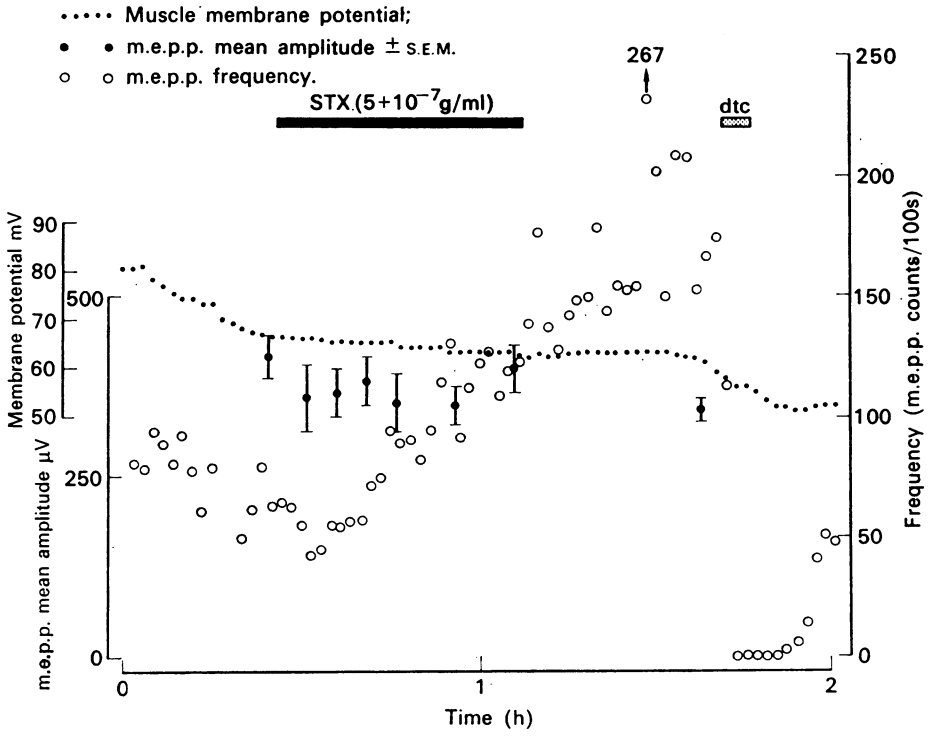

FIG. 1. Graph of muscle membrane potential, m.e.p.p. mean amplitude ± S.E.M. and m.e.p.p. frequency, recorded from an end-plate region of frog ext. dig. long. IV. muscle. The black bar indicates saxitoxin (5×10^{-7} g/ml) present in the irrigating Ringers solution. The shaded bar indicates addition of D-tubocurarine to the bath. Ordinate: time in hours. Right-hand abscissa: frequency expressed as m.e.p.p. counts/100 seconds. Left-hand abscissa: membrane potential (50–90 mV) and m.e.p.p. mean amplitude (0–500 μV).

either on the release of transmitter from the presynaptic terminals or on the excitation of ionic conductances brought about by the action of the transmitter on the postsynaptic receptors (Furukawa *et al.*, 1959; Elmqvist & Feldman, 1965; Katz & Miledi, 1967; Nishiyama, 1967).

There have been several studies of the actions of saxitoxin at the neuromuscular junction, but it was not until Kao & Nishiyama (1965) published their detailed study that it became clear that the end-plate was not readily affected by it, at least in moderate concentrations over a short period of time. They showed that the paralytic effects were caused either by a block of the propagated action potential in nerve or muscle, at low concentrations of saxitoxin (about 10^{-8} g/ml), which also reduced the amplitude of the e.p.p. by blocking conduction in the presynaptic terminals. The end-plate was depolarized when ACh was added to the bath fluid, even in the presence of a high concentration of saxitoxin, but it was not clear whether the sensitivity to acetylcholine was the same before and after exposure to the toxin. They reported that after adding saxitoxin (10^{-6} g/ml) to the bath m.e.p.p. frequency rose, but after prolonged exposure to the toxin the m.e.p.p. amplitudes fell.

Nishiyama (1967) tested the sensitivity of the end-plate to ACh by ionophoretic applications of ACh, during exposure to saxitoxin or tetrodotoxin. He found that they abolished the e.p.p without affecting the ACh potentials. However, he does not appear to have used concentrations of saxitoxin greater than 10^{-8} g/ml in these experiments, and the published records were of short duration. Nishiyama (1968) showed that prolonged exposure to saxitoxin (10^{-6} g/ml) did not significantly reduce m.e.p.p. amplitude, though there was a rise in frequency. He suggested that the reduction in amplitude seen by Kao & Nishiyama (1965) was caused by a deteriorating preparation. He mentions that during the earlier work the Ringers solution was not flowing through the muscle chamber when high concentrations of saxitoxin were being tested. Since the bath fluid was static during this part of Kao & Nishiyama's (1965) work, it is probable that deoxygenation, temperature or pH changes may well have occurred during the long period of exposure to the toxin, and may have been responsible for the late fall in m.e.p.p. amplitude.

The experiments reported in this paper indicate that saxitoxin has no significant effect on the sensitivity of the frog muscle end-plate. This confirms Nishiyama (1967), and extends his observations to include saxitoxin concentrations more than 100 times greater. It appears that saxitoxin could be used in place of tetrodotoxin in experiments on the receptors of the motor end-plate, and might have advantages over tetrodotoxin because of the ease with which it can be washed out.

These experiments on the m.e.p.p. have confirmed the findings of Kao & Nishiyama (1965) and Nishiyama (1968) who noted that m.e.p.p frequency often rose after exposure for several minutes to high concentrations of saxitoxin, but the reduction in amplitude reported by Kao and Nishiyama has not been confirmed. The reason for the rise in m.e.p.p. frequency is not clear.

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REFERENCES

DEL CASTILLO, J. & KATZ, B. (1955). On the localization of acetylcholine receptors. *J. Physiol., Lond.*, **128**, 157–181.

ELMQVIST, D. & FELDMAN, D. S. (1965). Spontaneous activity at a mammalian neuromuscular junction in tetrodotoxin. *Acta physiol. scand.*, **64**, 475–476.

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

HILLE, B. (1968). Pharmacological modifications of the sodium channels of frog nerve. *J. gen. Physiol.*, **51**, 199–219.

HILLE, B. (1970). Ionic channels in nerve membranes. In: *Progress in Biophysics and Molecular Biology*, Vol. **21**, ed. Butler, J. A. V. & Noble, D., pp. 1–32. Oxford: Pergamon Press.

KAO, C. Y. (1966). Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomena. *Pharmac. Rev.*, **18**, 997–1049.

KAO, C. Y. & NISHIYAMA A. (1965). Actions of saxitoxin on peripheral neuromuscular systems. *J. Physiol., Lond.*, **180**, 50–66.

KATZ, B. & MILEDI, R. (1967). Tetrodotoxin and neuromuscular transmission. *Proc. R. Soc. B.*, **167**, 8–22.

NISHIYAMA, A. (1967). Effect of saxitoxin on the end plate of frog muscle. *Nature, Lond.*, **215**, 201–202.

NISHIYAMA, A. (1968). Effect of saxitoxin on spontaneous release of acetyl-choline from the frog's motor nerve endings. *Tohoku J. exp. Med.*, **95**, 201–202.

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