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ACTIONS OF SAXITOXIN ON PERIPHERAL NEUROMUSCULAR SYSTEMS

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Recently several highly potent non-polypeptide neurotoxins of relatively low molecular weight have been purified from animal sources. Their chief action appears to be an interference with the production of action potentials in nerves and voluntary muscles, and in this they are some 100,000 times more potent than cocaine. *Tetrodotoxin* is obtained from the ovaries of the puffer fishes of the suborder Tetraodontidae; and *tarichatoxin* from the eggs of several California newts of the genus *Taricha*. *Saxitoxin* is extracted from the siphon of the Alaska butterclam, *Saxidomus giganteus*, and a closely related, probably identical, toxin is obtained from the hepatopancreas of several species of mussel, *Mytilus*. Although the ultimate source of saxitoxin is still uncertain (cf. Schantz, 1960), mussel toxin is known to be the product of an alga, *Gonyaulax*, that is ingested by the bivalve (Sommer, Whedon, Kofoid & Stohler, 1937). Shortly after their purification and crystallization the chemical identity of tetrodotoxin and tarichatoxin was established (Buchwald, Durham, Fischer, Harada, Mosher, Kao & Fuhrman, 1964), and the name tetrodotoxin has been assigned to designate them both (Mosher, Fuhrman, Buchwald & Fischer, 1964). The structure of tetrodotoxin is now known (Mosher *et al.* 1964) and its empirical formula is $C_{11}H_{17}N_3O_8$. *Saxitoxin*, and the related mussel toxin, although isolated in pure forms (cf. Schantz, 1960), have not yet been crystallized, and their structures are still not established; they are, however, known to be different from tetrodotoxin, since their empirical formula is $C_{10}H_{17}N_7O_4$ (Schantz, 1960).

Using a voltage-clamp technique on lobster giant axon preparations, tetrodotoxin was shown to have a specific and highly potent action in preventing the increase of sodium conductance normally associated with excitation (Narahashi, Moore & Scott, 1964; Takata, Moore, Kao & Fuhrman, 1965). The effects of tetrodotoxin in whole animals have been attributed to its axonal blocking action and the resultant diverse derangement of physiological functions (Kao & Fuhrman, 1963).

The actions of saxitoxin, and of the related mussel toxin, have been

studied to a lesser extent. With impure mussel toxin Fingerman, Forester & Stover (1953) showed that the compound action potentials in the frog sciatic nerve and in the innervated muscle were blocked; they also measured the contractions induced by acetylcholine and concluded (erroneously) that mussel toxin had a curarizing action. Bolton, Bergner, O'Neill & Wagley (1959) found that end-plate potentials were reduced in a frog nerve–muscle preparation. The blocking effects of pure shellfish toxins on the action potentials were demonstrated on frog nerve and on the electroplates of electric eel by Dettbarn, Higman, Rosenberg & Nachmansohn (1960), and on frog muscle by Evans (1964). In whole animals the cardiovascular, respiratory and neuromuscular effects have been studied by Prinzmetal, Sommer & Leake (1932), Kellaway (1935) and Müller (1935), using impure mussel toxin, and by D'Aguanno (1959) and Murtha (1960), using the pure toxin. In these studies, however, there was no attempt to correlate the effects in whole animals with the actions of the toxins at a cellular level, and the observations of the cellular actions were limited largely to seeing whether any interference with the action potential occurred. The objectives of the present experiments have been to study in greater detail the cellular actions of these toxins, which throw more light on the systemic effects. The actions of saxitoxin are compared with those of tetrodotoxin; since these toxins are known to be different, the finding that they have very similar biological actions has important implications in the further elucidation of the excitation phenomenon.

These results, parts of which have been presented in a preliminary form (Nishiyama & Kao, 1964; Kao & Nishiyama, 1965), were obtained independently of Evans's work (1964), published later.

METHODS

Toxins. Pure toxin powders obtained from clam (*Saxidomus giganteus*) and mussel (*Mytilus californiasus*), kindly supplied by Dr E. J. Schantz, were dissolved in 15% aqueous ethanol acidified to pH 3·0 with HCl. The stock solutions of 500 μg/ml. were stored at *ca.* 10° C and retained their potency for 9 months without evidence of deterioration. Dilutions were made from them either with Krebs's bicarbonate solution for mammalian experiments, or with one of the amphibian saline solutions for isolated frog tissues.

Since the molecular weight of saxitoxin dihydrochloride is 372 (Schantz, 1960), a concentration of 1 μg/ml. would be equal to 2·7 μM.

Systemic effects. Ten cats (3–6 kg) were anaesthetized with Na pentobarbitone, 30 mg/kg. The contractions of one tibialis anterior muscle evoked by stimulation of the ipsilateral sciatic nerve were recorded with a Grass FT 0·03 force transducer, and carotid blood pressure with a Statham P23AC pressure transducer. The response of one nictitating membrane to preganglionic stimulation of the cervical sympathetic nerve was also recorded, sometimes, with a Grass FT 0·03 force transducer. Drugs and toxins were injected via the femoral vein in volumes of 0·2–0·5 ml. Artificial respiration was not usually necessary after toxin, and only if neuromuscular paralysis occurred precipitously after large doses. The following drug salts were used: acetylcholine bromide, adrenaline bitartrate, atropine sulphate, D-tubocurarine chloride.

Isolated tissues. Sciatic nerves from frogs were excised, desheathed, and placed in a moist chamber. Records of propagated action potentials were taken on two pairs of Ag–AgCl electrodes with a condenser-coupled amplifying system, testing solutions being applied to the segment between the pairs of electrodes. After the blocking concentration range of the toxins was found, the nerves were also examined in a sucrose-gap apparatus (Stämpfli, 1954) with a direct-coupled system, so as to monitor changes in resting potential.

For end-plate activity, the sartorius-nerve preparations of frog were mounted in a 5 ml. chamber in which the bathing medium was recirculated by 95 % O_2 + 5 % CO_2 gas bubbles. Recording techniques, with intracellular micro-electrodes, were essentially the same as those of Fatt & Katz (1951). Current–voltage relations were also studied in the sartorius muscles in regions far removed from the end-plates, using two separate micro-electrodes, one for polarizing and one for recording.

Solutions. The isolated tissues of frog were usually bathed in a Conway solution containing (in m-mole/l.): 0·9 Ca^{2+}; 1·2 Mg^{2+}; 103·8 Na^{+}; 2·5 K^{+}; 74·5 Cl^{-}; 25·0 HCO_3^{-} 3·0 PO_4^{3-}; 1·8 SO_4^{2-}; 1·9 gluconate; and 26·0 glucose (Boyle & Conway, 1940). For studying end-plate activity, a modified saline solution of Fatt & Katz (1951) was often used, containing (in m-mole/l.): 3·6 Ca^{2+}; 113 Na^{+}; 2·0 K^{+}; 1 PO_4^{3-}; and 121 Cl^{-}. In most experiments in which end-plate potentials were studied D-tubocararine chloride in a concentration of 2 μg/ml. was incorporated into the saline solution to prevent muscle twitches. For studies on miniature end-plate potentials, this was omitted. In a few experiments in which miniature end-plate potentials and end-plate potentials were studied simultaneously, a solution containing 10 mM-Mg^{2+} but no D-tubocurarine was used (del Castillo & Katz, 1954).

The toxins were either added to a bath to give a final concentration of 0·001–1 μg/ml.; or, in the case of continuously flowing solutions, were incorporated in such concentrations in the test solution.

RESULTS

Similarity of mussel toxin and clam toxin (saxitoxin)

The term *shellfish toxins* will be used to designate both clam and mussel toxins, since no significant difference was observed between them in any of the present experiments.

Systemic effects

Effect of dose. The experiments of Wiberg & Stephenson (1960) show that the dose–response curve for shellfish toxins is very steep: the intraperitoneal LD_{50} in mice was *ca.* 7 μg/kg, and the LD_{99} *ca.* 13 μg/kg. Marked differences in systemic effects were also observed by us with slight changes in dose (Figs. 1, 2). The systemic effects produced by 2 μg/kg were the same as found by Kellaway (1935), D'Aguanno (1959) and Murtha (1960): there was a rapid and marked hypotension and severe neuromuscular paralysis (Fig. 1*a*). But 0·75 μg/kg produced marked paralysis without appreciable change in blood pressure (Fig. 2*b*, *d*) or heart rate. It is this hitherto undescribed dissociation of the two effects which constitutes practically the only observable difference between the actions of shellfish toxins and tetrodotoxin.

Neuromuscular systems. Both toxins (0·75–5 μg/kg) caused a weakening of tibialis anterior twitches (Figs. 1, 2). Depending upon the dose, the block was either partial, gradually reaching its maximum in 4–5 min, or

complete within 3–4 sec. When it was partial (but not when complete), a brief tetanic nerve stimulation always produced some relief (Fig. 2*b*, *d*) usually only for 10–20 sec, after which there was further paralysis, and rapid injections of acetylcholine (ACh) (0·5–1·0 mg) into the femoral artery always elicited a small contraction (Fig. 1*b*), indicating that some

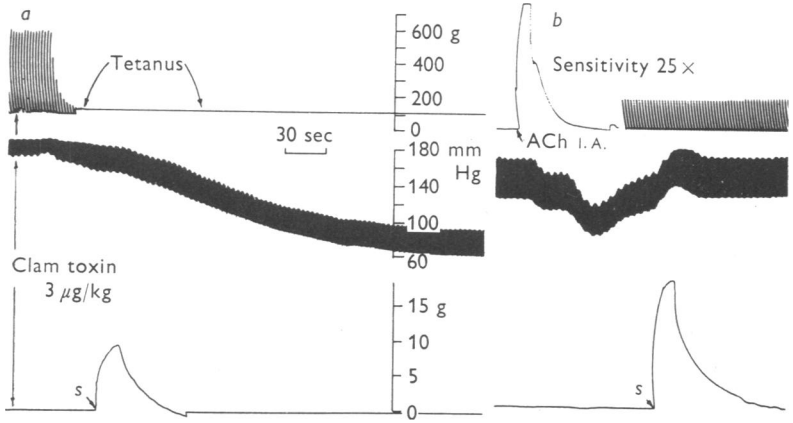

Fig. 1. Systemic effects of high doses of clam toxin in a cat (♂, 6 kg). See text. *Top trace*: tibialis anterior contractions on supramaximal sciatic nerve stimulation (1/sec). *Middle*: carotid blood pressure. *Bottom trace*: nictitating-membrane responses to preganglionic sympathetic stimulation (*S*). *a*, Effects of toxin 3 μg/kg. *b*, Partial block 3 hr 15 min later, after two further doses of toxin 0·75 μg/kg (and 5 min after atropine 0·2 mg/kg). Sensitivity increased (25×) only for the 0·5 mg ACh injection into the femoral artery.

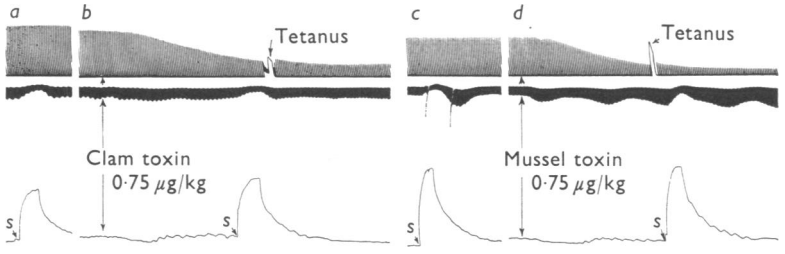

Fig. 2. Partial neuromuscular block without hypotension. Same experiment (and calibrations) as Fig. 1, beginning at (*a*) with full recovery 1 hr 40 min after the 3 μg/kg dose of Fig. 1*a*. (*b*) 0·75 μg/kg clam toxin; (*c*) almost full recovery 60 min later; (*d*) mussel toxin 0·75 μg/kg. Note post-tetanic potentiation in *b* and *d*. Artificial respiration was not required at any stage.

end-plates were still functioning. But after complete block ACh was effective for a short period only, and 1–2 min later it was ineffective. Some of these observations at first suggested a resemblance to curare, but further results indicate that this is not so, for example the micro-electrode studies

described below, which show that these toxins can render the muscle membrane inexcitable.

Respiratory muscles were more resistant to the toxins than tibialis, as also the responses to preganglionic cervical sympathetic stimulation (Figs. 1, 2).

Cardiovascular system. The hypotension appeared to be due primarily to a release of vasomotor tone resulting from paralysis of the sympathetic nerves, since vascular smooth muscles responded to adrenaline 4 μg/kg and electrocardiograms were normal (except after lethal doses), as also the responses of the heart to vagal stimulation, ACh and adrenaline.

Sensory endings. Paraesthesia and numbness occurred after application of toxin droplets (0·01 μg/ml.) to our lips and to the dorsum of the hand.

All the above phenomena could be due to a tarichatoxin type of axonal blocking action (Kao & Fuhrman, 1963), as is borne out below.

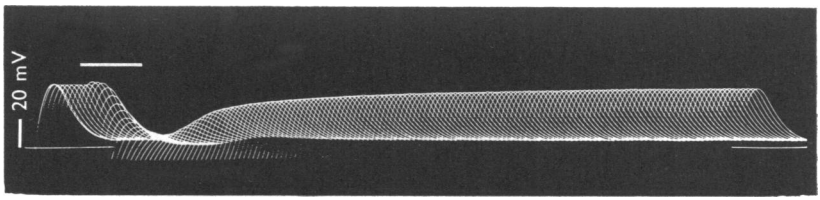

Fig. 3. Axonal blocking action of mussel toxin 0·01 μg/ml. Desheathed frog sciatic nerve in sucrose-gap apparatus; supramaximal cathodal stimulus every 4 sec; overlapping, successive compound action potentials, beginning left. Resting potential 62 mV, the base line being indicated by the two lower horizontal lines; total sweep time for each action potential 2 msec (not allowing complete return of repolarization phase to base line). Mussel toxin introduced after 10th impulse for 1 min (upper horizontal line). Note stability of resting potential, and slow but full recovery of action potential after toxin.

Nerve

Cellular effects

Both toxins (0·01 μg/ml.) produced complete block of the propagated compound action potential in desheathed frog sciatic nerves (Fig. 3); the minimum concentration producing appreciable block was 0·001 μg/ml. Characteristically, there was a reduction in the amplitude of the compound action potential without significant broadening, indicating that temporal dispersion of the conduction velocities in individual fibres was not involved. The block occurred without alteration of resting potential (Fig. 3). With 0·01 μg/ml. toxins it was completely reversible, and could be repeated as many as 8 times without permanent damage, provided complete recovery was allowed in between.

Neuromuscular junction

End-plate potentials. Clam toxin 0·01–0·1 μg/ml. always reduced the amplitude of end-plate potentials (EPP) in curarized frog nerve–sartorius preparations, after a delay of a few minutes, due possibly to diffusion lag. When the first sign of EPP reduction appeared, almost complete block followed within 30 sec (Fig. 4). Even up to the time of nearly complete block, the latency of EPP remained unchanged (Fig. 8). No significant depolarization accompanied the block (Fig. 5); its onset, duration and nature were essentially the same whether the medium contained 0·9 mM-Ca^{2+} and 1·2 mM-Mg^{2+} or 3·6 mM-Ca^{2+} and no Mg^{2+} (Fig. 4).

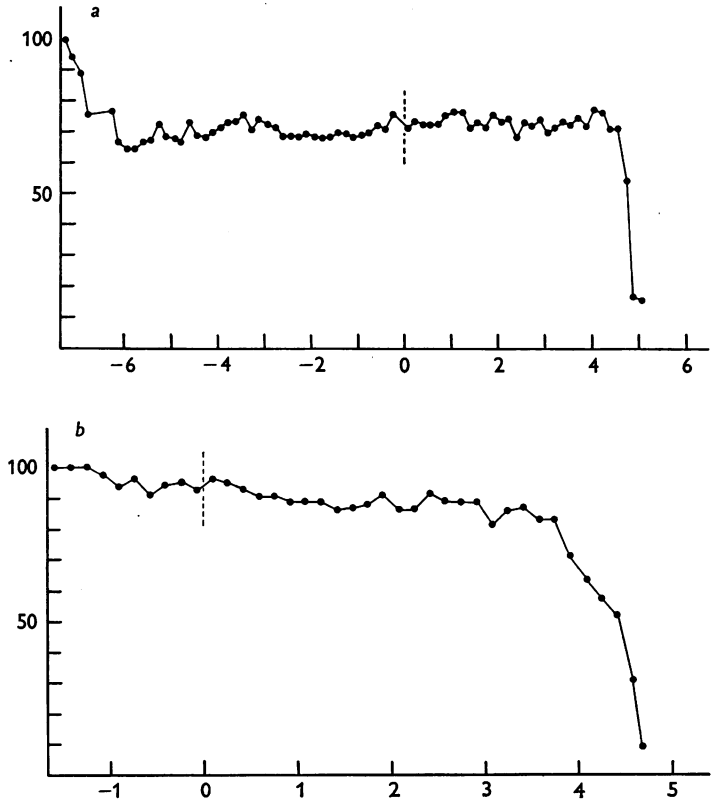

Fig. 4. Time course of block of end-plate potential by clam toxin 0·1 μg/ml. (*a*) and 1 μg/ml. (*b*); added at interrupted lines. Two different nerve–sartorius preparations. Control recordings before application of toxin show the slight decline (initial amplitudes 15·4 (in *a*) and 8·8 mV (in *b*) and range of fluctuation in EPP. Note lag of several minutes before rapid terminal block. (*a*) Frog saline containing 3·6 mM-Ca^{2+}, 1·3 μM D-tubocurarine Cl, no Mg^{2+}. (*b*) Conway solution containing 0·9 mM-Ca^{2+}, 1·2 mM-Mg^{2+}, 4·3 μM D-tubocurarine Cl.

During recovery from a blocked state there was often, on successive nerve stimulation, an irregular alternation of reduced EPPs with a complete absence of response (Fig. 8). The reason why this phenomenon was more easily observed in the recovery phase than during the onset of the block is probably a matter of concentration; it would seem to indicate an intermittent failure at the presynaptic nerve terminal.

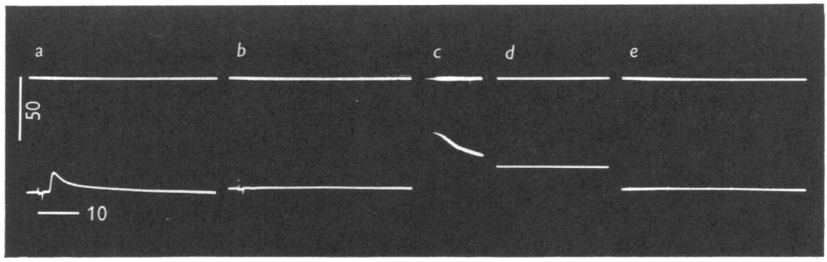

Fig. 5. Depolarization of toxin-blocked end-plate by acetylcholine (ACh). Frog nerve–sartorius preparation; Conway solution, D-tubocurarine 2 μg/ml. *Top line*, zero potential; *lower trace*, record from intracellular micro-electrode in one end-plate, the same throughout. Calibrations: 50 mV and 10 msec for all frames except *c* (where calibration = 2 sec). (*a*) Control: the transient depolarization is an EPP after a nerve stimulus. (*b*) Complete block of EPP by mussel toxin 0·1 μg/ml.; note membrane potential stability. (*c*) Response 30 sec after addition of ACh 20 μg/ml.; marked depolarization already present at beginning of record. (*d*) Partial recovery of membrane potential 5 min after ACh added. (*e*) Recovery of resting potential 1 min after saline washout; EPP still blocked.

Acetylcholine response of the toxin-affected neuromuscular junction. In single end-plates studied with micro-electrodes, when the EPP was abolished by either toxin, neither increased frequency of nerve stimulation nor eserine (10 μg/ml.) ever produced any relief of the block. On the other hand, when ACh 20 μg/ml. was added, a toxin-blocked end-plate was rapidly depolarized (Fig. 5). These observations indicate that the toxins did not produce a complete block of EPP by preventing ACh action at end-plate receptors; they indicate, rather, a failure of ACh release by the motor nerve. Further evidence that the block is primarily presynaptic, though not necessarily only at the nerve-ending, is given below.

Miniature end-plate potentials. To check on certain aspects of presynaptic activity, the frequency and amplitude of miniature end-plate potentials (MEPP) were monitored in uncurarized preparations, since the MEPP give an indication of the spontaneous discharge of transmitter from the nerve terminals (cf. del Castillo & Katz, 1956). The amplitude and the frequency of MEPP were highly variable at individual end-plates. The frequency of MEPP was less than 10/sec in most of the preparations, but in a few it was as high as 40–50/sec. Because of such variations from end-plate to end-plate, it was necessary to study the actions of drugs and

toxins always on the same end-plate. The general procedure was to record MEPPs of a single end-plate for a control period of *ca.* 10 min, then for 10 min or longer after the drug or toxin was applied, and for *ca.* 10 min after their removal.

An interesting effect of clam toxin 0·01–1 μg/ml. was an initial increase in MEPP frequency (Fig. 6), even in preparations that had an initial discharge rate higher than usual (Fig. 7). During this phase the amplitude of the individual MEPP did not appear to change. The frequency increased by 10–80 %. The duration of this stimulation phase lasted from a few minutes to 40 min. Thereafter there was a period of depression in which the frequency was appreciably below normal, and the amplitude of the individual MEPP reduced by *ca.* 40 % (Figs. 6*c*, 7). In contrast to the rapidity of block of EPP, the MEPPs were always present for at least 30–40 min, and up to 90 min after toxin.

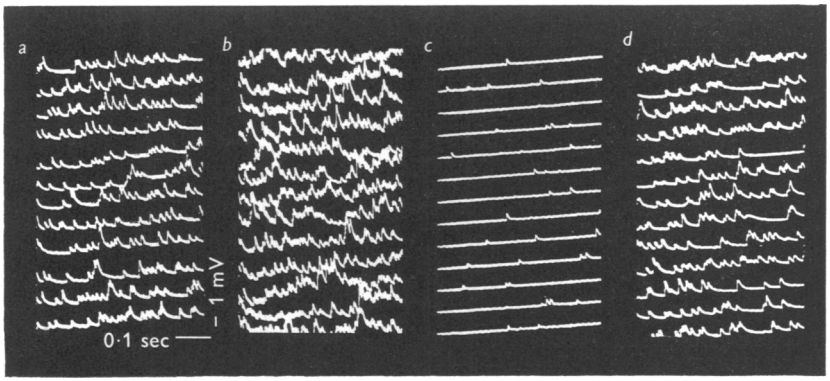

Fig. 6. Action of clam toxin on miniature end-plate potentials. All panels are excerpts of continuous records at one single end-plate; read from bottom to top. (*a*) Before toxin. (*b*) 10 min after application of clam toxin 1 μg/ml.; increased frequency and summation of individual MEPPs. (*c*) 80 min after toxin; reduction in amplitude and frequency. (*d*) Recovered state, 20 min after toxin washed out. The frequency of MEPP in the initial state was higher than usual, but in other preparations with lower frequencies similar changes were observed (see text).

The difference between the action of D-tubocurarine and of shellfish toxins is shown further by the following observation. D-Tubocurarine 0·3–4·0 μg/ml. reduced the amplitude of MEPPs without affecting their frequency; moreover, when MEPPs were blocked by D-tubocurarine, EPPs could still be elicited by nerve stimulation.

Relation between EPP and MEPPs in toxin-affected end-plates. In three experiments the EPP and MEPPs were monitored in the same end-plate. Since D-tubocurarine could not be used because of its interference with MEPPs, it was necessary to increase the magnesium to 10 mM, keeping the calcium at 1·8 mM (del Castillo & Katz, 1954). In all three experiments

after the application of clam toxin EPPs became quickly reduced in amplitude but MEPPs persisted (Fig. 8). In complete block EPP amplitude was reduced to that of individual MEPPs (Fig. 8*c*). Following a nerve volley there was neither a relief of the block nor any change in MEPPs. These experiments illustrate the difference between the effects of clam toxin on the EPP and MEPPs, and localize its action to a failure of conduction in the axon.

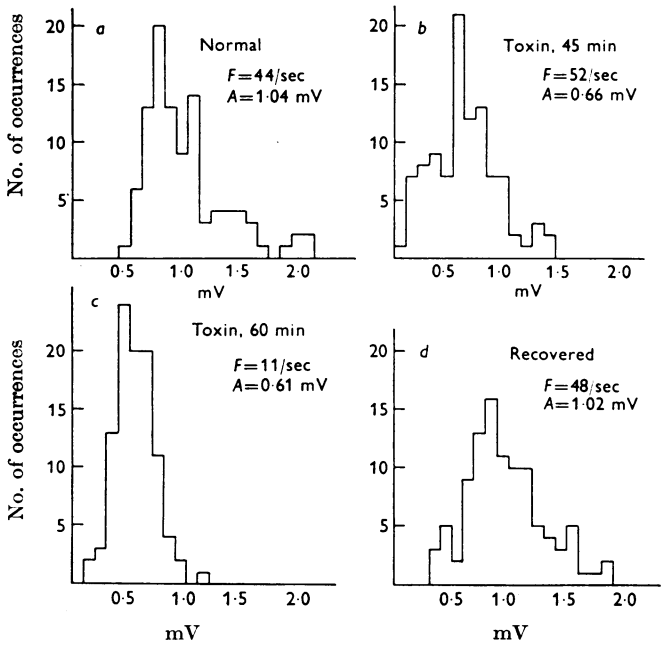

Fig. 7. Histogram of amplitudes of miniature end-plate potentials; preparation of Fig. 6. For each panel *F* represents mean frequency (rather higher than usual) and *A* mean amplitude of 100 random but continuous counts. Note that in the inhibitory phase of toxin action there is a shift of amplitude towards the low end; the stimulating phase could not be included because of summation of individual MEPPs, making it difficult to determine amplitude.

Muscle

Direct stimulation. When muscle fibres were stimulated through an intracellular micro-electrode, both toxins (0·01 μg/ml.) increased the threshold potential at which a propagated spike arose and reduced the amplitude of the spike height (Fig. 9*b*, *c* and Table 1). In Fig. 9*b* the largest response was 83 mV, which was probably propagated because the muscle fibre was seen to contract. With 0·05–1 μg/ml. propagation was suppressed and microscopic observation showed that the contractions had become strictly localized to the impaled area. The electrical responses to

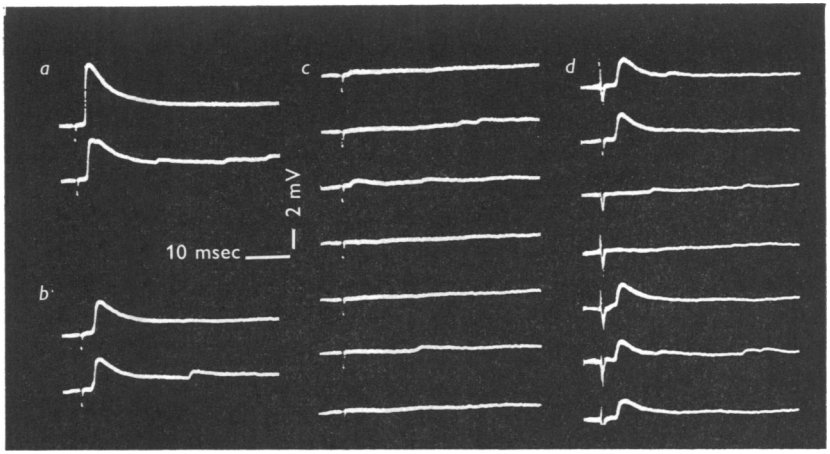

Fig. 8. Relation between EPP and MEPP in toxin-treated preparations. Frog nerve–sartorius preparation in saline containing 10 mM-Mg^{2+}, no D-tubocurarine. In all records EPP is the large transient depolarization immediately following the stimulus artifact, and MEPP the small random depolarizations in the traces. (*a*) Control. (*b*) 6 min 20 sec after application of clam toxin 1 μg/ml; reduced amplitude of EPP. (*c*) 8 min after toxin; EPP completely blocked but MEPPs still present. (*d*) Recovery, 30 min after toxin washed out. Note intermittent block of EPP. All records are excerpts from continuous records; read from bottom to top.

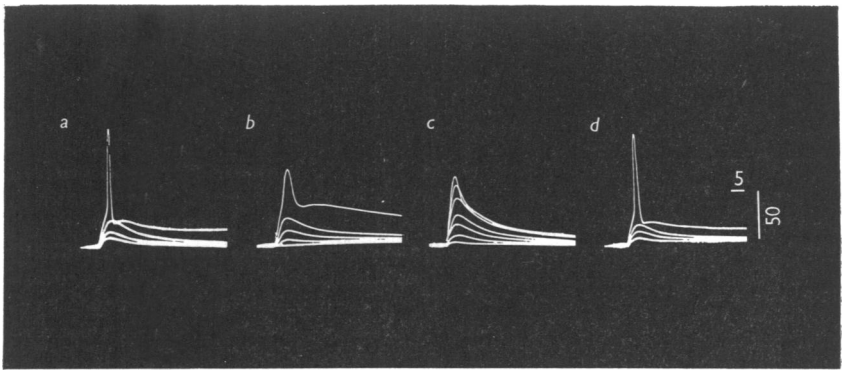

Fig. 9. Blocking by clam toxin of spike production in frog sartorius muscle fibre (Conway solution). (*a*) Normal responses to direct stimuli of varying strengths through an intracellular micro-electrode. (*b*) 60 min after addition of clam toxin 0·01 μg/ml.; largest depolarization, 83 mV; trace shift following spike is caused by contraction dislodging micro-electrode. (*c*, *d*) from another preparation: (*c*) 60 min after addition of clam toxin 0·1 μg/ml. Note graded nature of response and faster repolarization of largest response (72 mV). Only localized contractions were observed in this state. (*d*) Recovery 60 min after toxin washout. Calibration: 5 msec, 50 mV.

brief cathodal pulses then assumed shapes similar to certain large non-propagating local responses observed in heart muscles under conditions in which the regenerative spike-producing mechanism was inoperative (Kao & Hoffman, 1958). The amplitude of these responses could be graded by varying the intensity of the stimulus, and a response as large as 72mV (Fig. 9*c*) did not initiate a spike. These observations indicate that the toxin probably interfered with the regenerative portion of the increase in sodium inward current which normally leads to spike production (Hodgkin & Huxley, 1952). That the potassium conductance of the membrane probably was not affected could be seen in Fig. 9*c*, in which the rate of repolarization of the largest response was faster than those for the smaller responses. This increased rate was most likely caused by delayed rectification of the membrane, which has been attributed to an increase in potassium conductance normally following a depolarization (Katz, 1949; Hodgkin & Huxley, 1952; Jenerick, 1959; Adrian & Freygang, 1962).

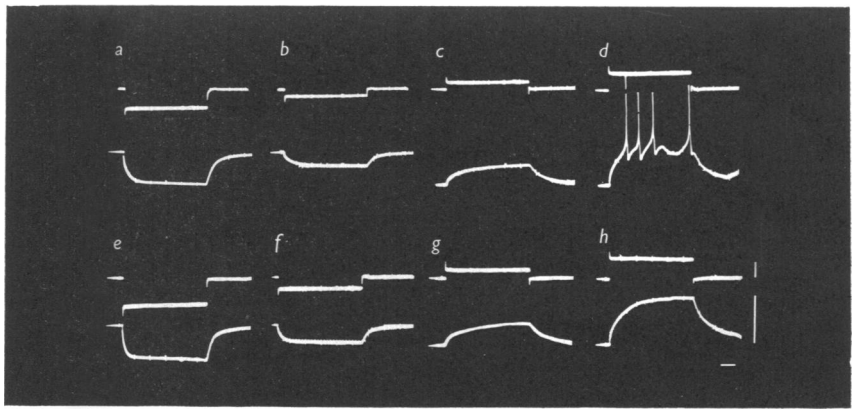

Fig. 10. Current–voltage records in normal and toxin-treated frog sartorius fibre (Conway solution); two micro-electrodes used. In all records upper trace shows applied current pulses and lower trace membrane potential changes produced by the applied current. Deflexions downward indicate inward current and hyperpolarization; upward deflexions, outward current and depolarization. (*a–d*) Normal muscle showing repetitive spikes at threshold depolarization. (*e–h*) Same fibre 60 min after treatment with clam toxin 0·1 μg/ml. No spikes even with large outward current pulse (*h*). Calibrations in lower right corner applicable to all frames: 10^{-7} A for current trace, 50 mV for voltage trace, and 5 msec.

Current–voltage relation. These experiments were performed with the tips of the polarizing and recording micro-electrodes separated by $< 100\,\mu$. Since the distance is small compared to the length constant of frog muscle fibre (2–2·4 mm), the steady state potential caused by a current pulse follows the simplified relation $V = \frac{1}{2} I \sqrt{(r_m)(r_i)}$ (Fatt & Katz, 1951), and $V/I = \frac{1}{2}[\sqrt{(r_m)(r_i)}]$ is the effective resistance. When the internal resistance

r_i and the fibre diameter remain constant, changes in V/I reflect changes in membrane resistance r_m.

The actual records of such an experiment are shown in Fig. 10 and the analysed results from two other pairs of muscle fibres are shown in Fig. 11. In these preparations anomalous rectification (Katz, 1949; Adrian & Freygang, 1962) was evident in both the normal and the toxin-treated preparations. With inward current pulses, the effective resistance was *ca.* 20 % lower at 80 mV hyperpolarization than at 5 mV; and, with outward current pulse, it was *ca.* 30 % higher at 30 mV depolarization than at 10 mV. Clam toxin did not materially alter these ranges.

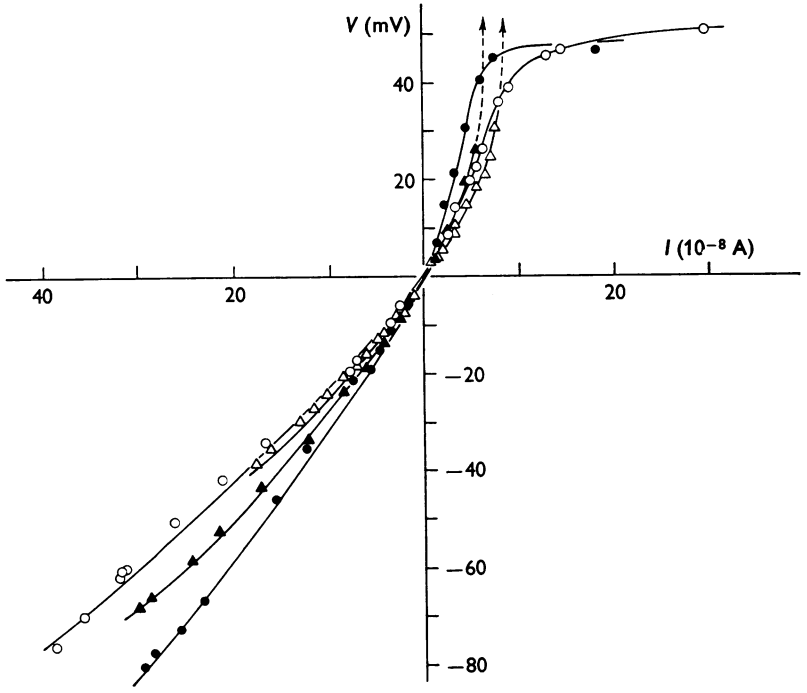

Fig. 11. Current–voltage relation in normal and toxin-treated muscle fibres. Two frog sartorius preparations in Conway solution. Hollow symbols for one pair and filled symbols for the other. Triangles, normal preparations; circles, clam toxin, 0·1 μg/ml. Depolarization upwards, hyperpolarization downwards; outward current right, inward current left. Note curvature up to 30 mV depolarization indicating anomalous rectification, and delayed rectification in toxin-treated fibres. Normal fibres gave rise to spikes indicated by arrows.

The most striking difference in the current–voltage relation of normal and toxin-treated muscle fibres was seen with strong outward current pulses. In the untreated fibres, pulses larger than 5×10^{-8} A elicited spikes and if maintained produced repetitive spikes (Fig. 10*d*). In the toxin-

treated fibres, outward current six times stronger did not produce spikes (Figs. 10*h* and 11). In the record shown in Fig. 10*h* there was no change in the voltage response indicative of delayed rectification, but in other experiments it was readily observed. However, the presence of delayed rectification which lowered membrane resistance could be clearly seen in the toxin-treated fibres, since these fibres could pass large outward current with little change in voltage (Fig. 11). As in normal muscle fibres (Jenerick, 1959; Adrian & Freygang, 1962), this phenomenon first became evident at 20–30 mV depolarization. The results in Fig. 11 are in agreement with those on normal muscle fibres in which spike production had been prevented by removal of external sodium (Adrian & Freygang, 1962). From this it can be concluded that the toxins did not alter the increased potassium conductance responsible for delayed rectification. Lastly, since the effective resistance is not significantly increased by toxin treatment (Table 1), and since there is no reason to suspect that either the fibre diameter or the internal resistance had been changed, it can be concluded that both the potassium and the chloride conductances were essentially unaffected.

TABLE 1. Effect of saxitoxin on frog sartorius muscle fibre

Muscle	Condition	Resting potential (mV)	Threshold potential (mV)	Spike (mV)	Effective resistance (kΩ)
1	Normal	91·8 (16)	35·2 (17) Range 32–40	125	—
2	Normal	103 (1)	35 (1)	—	263 (1)
	Saxitoxin 1 μg/ml.	88·3 (3)	—	None	315 (1)
3	Saxitoxin 0·1 μg/ml.	97·8 (8)	—	None	—
4	Saxitoxin 0·05 μg/ml.	105 (11)	—	None	—
	Recovered	97·8 (16)	36·9 (8) Range 32–46	120	—
5	Saxitoxin 0·01 μg/ml.	95·4 (7)	49·7 (7) Range 46–53	99 Range 88–99	—
	Recovered	95·0 (4)	34·7 (7) Range 32–38	135	—
6	Saxitoxin 0·1 μg/ml.	83·9 (7)	—	None	469 (2)
7	Saxitoxin 0·1 μg/ml.	92·5 (12)	—	None	294 (5)
	Recovered	95·1 (25)	42 (5) Range 38–48	110	302 (4)
8	Normal	92·0 (20)	40 (6) Range 36–43	116	271 (8)
				Mean (kΩ): Normal 280, Toxin 340	$P > 0{\cdot}3$

All values except those for spike are mean values. Numbers in parentheses indicate size of samples. Values for spike are maximum values.

DISCUSSION

Systemic effects. The peripheral actions of these shellfish toxins would account adequately for the paralysis, hypotension and respiratory failure, but concomitant central effects upon the respiratory and vasomotor centres are not excluded (cf. Kellaway, 1935).

Assuming a lethal concentration in the body water of 0·01 μg/ml. the lethal dose for a human adult would be *ca.* 0·4 mg I.V.; orally, Meyer (1953) estimated it as 8 mg.

Cellular actions. The present results indicate that, like tetrodotoxin and tarichatoxin (Kao & Fuhrman, 1963, and references quoted therein), clam and mussel toxins are potent blocking agents of excitation. The neuromuscular block produced by them is quite different from that caused by either competitive (e.g. D-tubocurarine) or depolarizing and desensitizing (e.g. decamethonium) blockers. This conclusion is based on the observations that neither repetitive nerve stimulation nor eserine reversed the block, and that at the end-plate acetylcholine (ACh) in large amounts still produced a marked depolarization. Since after toxin the EPP disappeared long before MEPPs, the block would appear to be due to an earlier failure in the motor axons rather than at the nerve endings. The constancy of the latency of EPP until block is complete suggests that the ACh-releasing mechanism at the nerve endings was not markedly affected. On the postjunctional side, since toxin interfered with the excitation of the muscle fibres a recording of only the contractile or even electrical activity of the whole muscle could lead to the erroneous conclusion that the ACh response of the end-plates was depressed (Kellaway, 1935; Fingerman *et al.* 1953; and concurring opinion of Evans, 1964). The present results do not categorically exclude an action at the end-plates. In fact, a decrease in the amplitude of MEPPs suggests that some interference could be present; the important point, however, is that it constitutes only a minor part of the block. The preponderant action of these toxins is exerted on those membranes that respond with regenerative spike processes, i.e. on the axons and on the muscle membrane.

On such membranes the actions of shellfish toxins are similar to those of tetrodotoxin, viz. a specific interference with the increase in sodium conductance normally associated with excitation (Narahashi, Deguchi, Urakawa & Ohkubo, 1961; Narahashi *et al.* 1964; Takata *et al.* 1965). This conclusion is based on the observations that the potassium and chloride conductances were unaffected while the consequence of the normal increase in sodium conductance upon threshold depolarization, the spike, was effectively reduced and abolished. A detailed analysis of the dynamic aspects of the alterations in sodium conductance must, however, be made by the voltage-clamp technique, as has been done for tetrodotoxin (Narahashi *et al.* 1964) and tarichatoxin (Takata *et al.* 1965).

Comparison of saxitoxin and tetrodotoxin. Although the actions of these toxins are remarkably similar, there is one outstanding difference in their effects on the blood pressure: with low, paralytic doses of the shellfish toxins, there is no significant hypotension; with tetrodotoxin, hypotension

always accompanies muscular paralysis, whatever the dose. Clinically hypotension is uncommon in paralytic shellfish poisoning (Meyer, Sommer & Schoenholtz, 1928; Sommer & Meyer, 1937) but is an early sign portending grave prognosis in puffer fish poisoning.

Despite the established chemical non-identity of shellfish toxins and tetrodotoxin the similarity in their biological actions suggests a resemblance in chemical structure; in fact both are known to contain guanidinium groups (Mosher *et al.* 1964; Schantz, 1960) and they may therefore possibly have an identical type of action at the molecular level.

Possible mechanism of action. That guanidinium compounds have a unique action on the excitation process is already known (Larramendi, Lorente de Nó & Vidal, 1956): their effects on ionic mechanisms of excitation have been studied by Lüttgau (1958) and Deck (1958).

Possibly the site in the membrane where sodium moves downhill during the spike also admits guanidinium groups with their strong positive charge. Thus, with guanidine itself, positive charges are carried inwards, resulting in its ability to replace sodium as the current carrier (Lüttgau, 1958). But, with certain substituted guanidine compounds, movement through the sodium channel is impaired so that the spikes restored by them have only slow rising phases (Deck, 1958). In the cases of tetrodotoxin and saxitoxin, however, while the guanidinium groups may enter the sodium channels, the remainder of the molecule may prevent passage of the toxin through them, effectively blocking these sites to sodium and leading to prevention of impulse generation. To account for the high potency and selectivity of action of these toxins, the structure of the membrane around the sodium channels may be complementary to parts of the toxin molecule in stereochemical proximity to the guanidinium groups. If this scheme is plausible, then it is hoped that these potent toxins or some of their analogues may prove useful for the investigation of the molecular architecture of certain crucial membrane areas, or for the development of a new class of local anaesthetic.

SUMMARY

1. Purified clam toxin (saxitoxin) and mussel toxin appear identical in their actions in whole animals and on isolated nerves, skeletal muscle fibres, and end-plates.

2. In doses of 0·75 μg/kg they produced marked weakening of nerve-elicited twitches in tibialis anterior without producing hypotension.

3. In higher doses (*ca.* 2 μg/kg) hypotension accompanied the paralysis and was probably due to reduced sympathetic tone.

4. Both toxins 0·01 μg/ml. (27×10^{-9} M) rapidly and reversibly blocked propagated action potentials in desheathed frog sciatic nerve. No depolarization was associated with the block.

5. The neuromuscular block produced by the toxins (0·01–1 μg/ml.) was not accompanied by end-plate depolarization. It was characterized by a block of EPP that was not reversed by repetitive nerve stimulation or eserine. Acetylcholine readily depolarized a toxin-blocked end-plate. MEPPs also persisted long after EPPs were blocked. Hence, it was concluded that the block was in the motor axon and not at the end-plate receptors.

6. With doses of 1 μg/ml. there was an initial increase in MEPP frequency, followed by a decrease. Some reduction in MEPP amplitude occurred when the frequency was depressed.

7. With 0·1 μg/ml. skeletal muscle fibres were rendered incapable of producing spikes although the resting potential was maintained. This inexcitability persisted even with large outward currents.

8. Delayed and anomalous rectification in muscle fibres appeared unaltered by the toxin. Membrane resistance as inferred from the current–voltage relations remained the same.

9. The conclusion is drawn that at a cellular level these toxins acted by specifically blocking the increase of sodium conductance normally associated with excitation, without affecting the potassium conductance or changes therein during excitation, nor the chloride conductance.

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