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THE EFFECT OF TETANUS TOXIN IN THE GOLDFISH

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SUMMARY

1. The effects of tetanus toxin have been investigated both on central nervous and peripheral neuromuscular systems of goldfish.

2. Tetanus toxin kills goldfish when administered in minute doses. The lethal effect is temperature dependent. Unlike mammals, in which tetanus toxin produces spastic paralysis and convulsions, the tetanus-intoxicated goldfish die with an apparently flaccid paralysis.

3. Inhibition and excitation were investigated on the Mauthner cells of goldfish which had been paralysed with tetanus toxin for at least 24 hr, and which were subsequently kept alive for up to 3 days by perfusing the gills with oxygenated water. In such fish, antidromically and orthodromically excited Mauthner cells were quite normal, and there was no apparent effect on either the collateral inhibition or the crossed VIIIth nerve inhibition.

4. Local injection of sublethal doses of tetanus toxin into the pectoral fin muscles produced local paralysis of the fin. Nerve–muscle preparations were isolated from such goldfish; in tetanus toxin-paralysed fins, the muscle no longer responded to stimulation of its nerve. The nerve compound action potential was still present and the muscle still responded vigorously to direct electrical stimulation.

5. It is concluded that the major part of the lethal action of the toxin in the fish must be ascribed to a peripheral effect, the blocking of neuromuscular transmission. The inhibitory neuronal systems acting on the Mauthner cells of the goldfish, in apparent contrast to those acting on mammalian spinal neurones, are highly insensitive to tetanus toxin.

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INTRODUCTION

Botulinum and tetanus toxins, produced by the anaerobic sporing bacilli, *Clostridium botulinum* and *Cl. tetani* respectively, are both potent neurotoxins. It is generally considered that while botulinum toxin paralyses animals by blocking neuromuscular transmission, the primarily convulsive effects of tetanus toxin are due to a blocking of inhibition in the central nervous system (see Eccles, 1964). Botulinum toxin acts presynaptically at the neuromuscular junction, preventing the release of acetylcholine in response to nerve stimulation, and the quantal release which occurs spontaneously (Ambache, 1949; Brooks, 1956). The effect of tetanus toxin, however, is not nearly so well worked out. Brooks, Curtis & Eccles (1957) found that the toxin, like strychnine, increased the polysynaptic reflex responses in cats, leaving monosynaptic ones unaffected, and that it decreased five different types of inhibition which were investigated. The electrical responses on the surface of the spinal cord believed to result from activity in inhibitory interneurones were apparently unaffected, and it was suggested that the toxin probably exerted its effects at the inhibitory synapses on the motoneurones. Curtis & de Groat (1968) recorded Renshaw cell activity extracellularly, before and after the application of tetanus toxin from a micropipette situated close to the cell; the toxin prevented the inhibition (produced by squeezing the ipsilateral hind paw) of acetylcholine-induced and spontaneous Renshaw cell firing. In contrast, the inhibition of Renshaw cells by iontophoretically applied glycine (a putative inhibitory transmitter substance) was not blocked by tetanus toxin. Curtis & de Groat therefore suggested that tetanus toxin could be acting presynaptically at inhibitory synapses (but see Roper, Diamond & Yasargil, 1969).

The various mechanisms of synaptic transmission at the Mauthner cell of the goldfish have been analysed in considerable detail (Furshpan & Furukawa, 1962; Furukawa & Furshpan, 1963; Furukawa, 1966; Diamond, 1968). The cell can be relatively easily identified electrophysiologically, and micro-electrodes can be located with some accuracy in the morphologically distinct synaptic regions. The present study was initially undertaken to investigate the possible effects of tetanus toxin on physiological and pharmacological inhibition of Mauthner cells. However, although in preliminary experiments even minute doses of the toxin killed the fish, no abnormalities were detected in the various responses of these neurones even in acutely intoxicated fish. The fish died with an apparently flaccid paralysis associated with the cessation of respiration.

Now although the suppression of synaptic inhibition in the central nervous system is commonly accepted as responsible for the convulsions

and spasticity in tetanus-intoxicated animals (including birds (Davies, Morgan & Wright, 1955), reptiles (Cowles & Nelson, 1947) and frogs (Rowson, 1961)), there is evidence even in mammals for another, peripheral action of tetanus toxin, comparable to the neuromuscular blocking action of botulinum toxin (Kaeser & Saner, 1969; see also Ambache, Morgan & Wright, 1948*a*, *b*; van Heyningen & Mellanby, 1970). The apparently flaccid paralysis produced in goldfish, coupled with the apparent insensitivity of the central nervous system to the toxin, pointed strongly to a peripheral action of the toxin in fish.

The work reported here attempts to separate the possible central and peripheral actions of tetanus toxin. The results suggest that goldfish are particularly suitable for the study of the peripheral action of the toxin and may be relatively insensitive to its central effects. A preliminary report of these studies has been published (Diamond & Mellanby, 1970).

METHODS

Recording excitation and inhibition in the Mauthner cell

The methods are essentially those described by Furshpan & Furukawa (1962), Furukawa & Furshpan (1963) and Diamond (1968). The fish (*Carassius auratus* L.) contained in a special chamber, was perfused continuously through the mouth with water containing as an anaesthetic 0·25–0·3 % urethane. The Mauthner axons were excited by a pair of steel wires, insulated except at the tip, which were positioned just outside the vertebral column. The VIIIth nerves were excited by a similar pair of electrodes inserted through holes in the skull. Mauthner cell activity was recorded with conventional glass KCl-filled micro-electrodes. The late collateral inhibition (L.C.I. of Furukawa & Furshpan, 1963) was revealed either as a hyperpolarization recorded in the Mauthner cell following the antidromic spike, and/or by the reduction in size of a second antidromic spike produced within 4–7 msec of the first. (This reduction is a useful measure of the post-synaptic inhibitory conductance change.) This inhibition characteristically disappears when the pair of stimuli are delivered at 5–10/sec, due to fatigue, and in this way its effect on spike height can be distinguished from any reduction of spike height due to refractoriness. The crossed VIIIth nerve inhibition was also measured either as a hyperpolarization of the Mauthner cell or by the reduction it caused in the amplitude of the antidromic spike.

The isolated nerve-muscle preparation

Fig. 1 shows the muscle which was used in the experiments *in situ* and the preparation as it was dissected out. It was mounted on a cork platform with a pin through the remaining piece of pectoral girdle, so that the *adductor superficialis* muscle hung vertically. A loop was secured through the base of the fin and attached to a fine chain suspended from the arm of a tension transducer. The output from the transducer was fed directly into a cathode ray oscilloscope. The nerve was stimulated with a pair of chlorided silver wire electrodes. Direct stimulation of the muscle was done by placing electrodes across it at the base and at the fin end, so that they made contact with as much of the surface as possible. The whole preparation was immersed in continuously oxygenated Ringer solution (NaCl 132 mM; KCl 3·1 mM;

$CaCl_2$ 3 mM; $MgCl_2$ 1 mM; Na-phosphate buffer 3 mM, pH 7; glucose 5·5 mM) except when being stimulated; such preparations could remain responsive for 24–48 hr after isolation from the fish. The muscles taken from fish weighing 60–80 g (length excluding tail, 14–20 cm) gave maximal twitch tensions of 5–20 g, and tetanic tensions of 10–40 g.

The compound action potential recorded from the electrically excited nerve to the fin muscle was displayed after amplification, on the screen of an oscilloscope which was photographed.

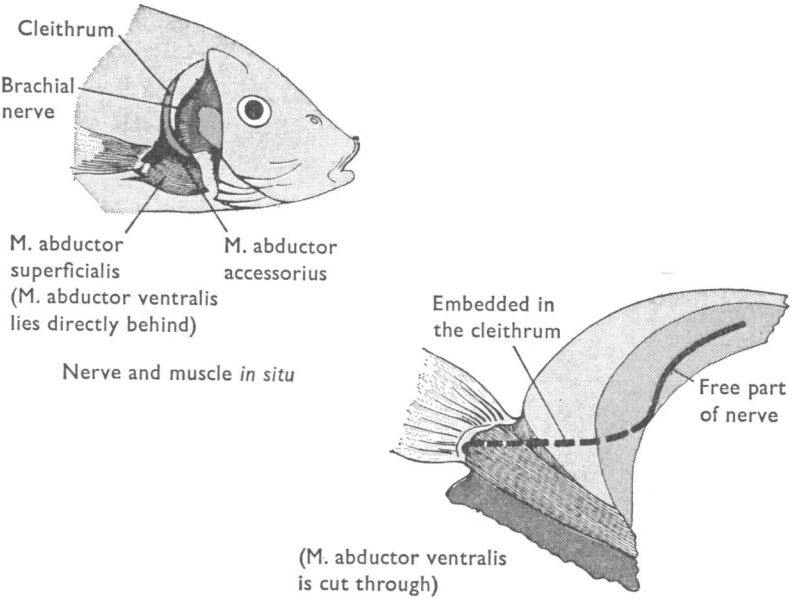

Fig. 1. The nerve–muscle preparation.

Tetanus toxin. The preparation, which was kindly provided by Dr R. O. Thomson of the Wellcome Research Laboratories, contained 60 million LD_{50}/mg and 360 L+ doses/mg. It was 41 % protein, the rest being made up of salts. The toxin was assayed by injecting serial twofold dilutions of the experimental solutions into pairs of Swiss albino mice. One LD_{50} was defined as the amount of toxin which would kill half the mice injected with it within 7 days.

Tetanus antitoxin solution (also obtained from Dr Thomson) contained 1800 Lf/ml.

Injections of fish. The preparations to be injected were dissolved in fish-Ringer solution containing 0·2 % gelatin as protective colloid. Injections were made at the base of the tail, into the belly of the pectoral fin muscles, or directly over the brain through a hole drilled in the skull.

RESULTS

Toxicity of tetanus toxin to goldfish

The toxicity of intramuscularly injected tetanus toxin, as measured either by the time to death following a given dose, or by the dose needed to

kill within a selected period, depends on the temperature of the goldfish. Fig. 2 shows the effects of temperature on the toxicity (as measured by time required to kill) of various doses of tetanus toxin. At 28° C, 20 mouse LD_{50} of tetanus toxin killed the fish within 5 days, whereas at 18° C this dose of toxin had no apparent effect on the fish within 50 days.

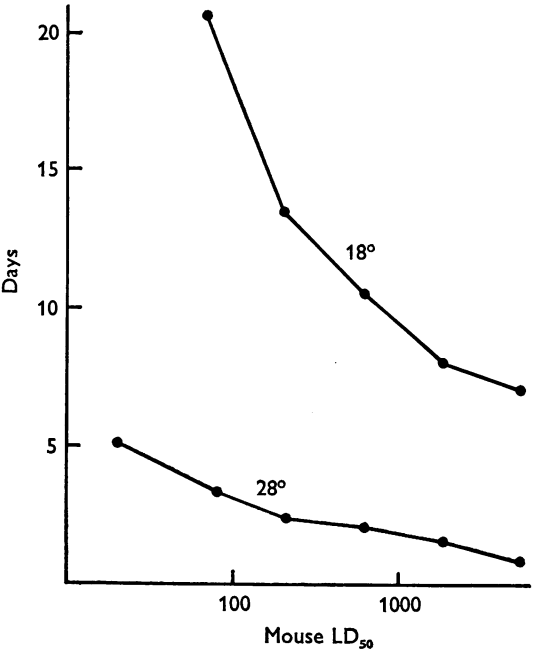

Fig. 2. Toxicity of tetanus toxin in goldfish. The abscissa shows (on a log scale) the dose of the tetanus toxin (measured in mouse lethal doses, LD_{50}) given intramuscularly; the ordinate shows the time taken for the fish to die. The two series of results were obtained from fish kept at the two indicated temperatures (18 and 28° C).

Symptoms of tetanus intoxication in goldfish

General. When fish had been injected (either at the base of the tail, or over the brain) with a 'lethal' dose of tetanus toxin, the earliest change in behaviour was occasional erratic swimming, particularly in response to a bang on the tank. During the following 2–3 days the fish became increasingly lethargic, and the respiratory movements of the operculi became spasmodic and less frequent; eventually the animals turned on to their sides and floated immobile. At this stage they sometimes responded to a bang on the side of the tank by normal, or sometimes erratic, swimming for a period of a few seconds. About 6–7 days after injection, breathing movements ceased

and they became completely paralysed. The heart often continued to beat for a few hours, but the animals always died within a day.

Local. If a single small dose (10–200 mouse LD_{50}) of tetanus toxin was injected into the pectoral fin muscles, an apparently localized effect of tetanus toxin was produced. After about 4 days (with 20 LD_{50}) at 18° C it was noticeable that the fin was held closer to the body than normal. Over the next few days it became progressively paralysed. Usually the fin was flaccid, but occasionally the muscle seemed to be stiff, and then the fin presented detectable resistance to passive movement, even when its motor nerves were cut. It was still, however, responsive to *direct* electrical stimulation (see below).

Investigations on the central nervous system of goldfish intoxicated with tetanus toxin

Initially the Mauthner cell was investigated in intoxicated fish whose swimming was markedly abnormal and erratic. Fig. 3 shows records obtained from one of the Mauthner cells of such a fish. In *A* the 'late collateral inhibition' or LCI (Furukawa & Furshpan, 1963) is demonstrated. Two antidromic spikes were fired, with an interval of a few msec between them; the second spike of the pair was markedly reduced in

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Legend to Fig. 3.

Inhibition of the Mauthner cell in intoxicated goldfish. A. The late collateral inhibition. Each record (upper and lower) shows two antidromically-excited spikes recorded intracellularly from one Mauthner cell. In the upper record a single pair of antidromic stimuli only was used. The lower record shows a number of superimposed traces like that above, but the pair of stimuli was repeated at 10/sec (the first few traces of the series were not photographed). The collateral inhibition is revealed as a reduction in amplitude of the second spike of the pair and is present in the upper record, but 'fatigued-out' in the lower (see text for further description).

B. The crossed VIIIth nerve inhibition. Upper record, single antidromically-excited Mauthner cell spike. Lower record, the same spike but now preceded by a stimulus to the opposite VIIIth nerve (the now reduced spike is superimposed on a slow positivity; see Furukawa & Furshpan, 1963).

C. VIIIth nerve excitation and inhibition. On the left, the Mauthner cell spike fires from an e.p.s.p. which is indicated by the arrow, and which follows the large stimulus artifact. On the right, the same orthodromic excitation is preceded by a stimulus to the opposite VIIIth nerve (compare *B* above). The spike is absent, and a subthreshold e.p.s.p. is left (marked by the arrow).

Calibrations: vertical: 5 mV (*A*, *B* and *C*); horizontal: 1 msec (*A* and *C*); 0·5 msec (*B*).

amplitude (Fig. 3*A*, upper record). This reduction is due to the activation of the collateral inhibition, and is the consequence of the reduced input resistance of the Mauthner cell (i.e. the reduction in spike amplitude reveals the inhibitory conductance increase of the post-synaptic membrane). In the lower record the pair of stimuli were repeated 10 times a second, at which frequency the inhibition fatigues; the two antidromic

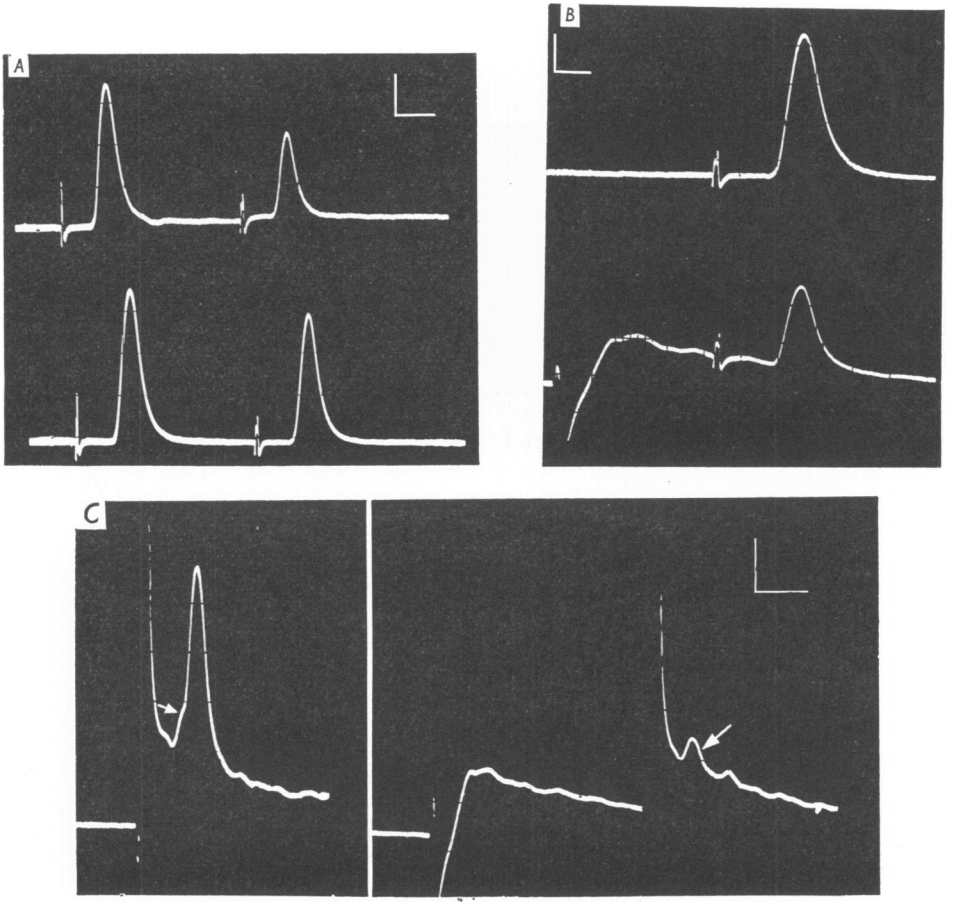

Fig. 3. For legend see opposite page.

spikes were now similar in size. (The small reduction in the second spike was due to refractoriness of the spike-generating mechanism.) Fig. 3*B* demonstrates a similar reduction in the height of an antidromic spike, in this instance when it occurred a few milliseconds after stimulation of the opposite VIIIth nerve; the VIIIth nerve was shown by Furukawa & Furshpan (1963) to exert predominantly an inhibitory influence on the

contralateral Mauthner cell. Fig. 3*C* shows this crossed VIIIth nerve inhibition acting on an *orthodromically*-excited Mauthner cell, i.e. fired by a stimulus applied to the *ipsilateral* VIIIth nerve. The first record on the left shows the orthodromic spike alone; in contrast to the antidromic spike it is initiated by a clearly defined excitatory post-synaptic potential (e.p.s.p.). The second record shows the inhibition caused by excitation of the opposite VIIIth nerve. In this instance the contralateral VIIIth nerve inhibition was very powerful: the Mauthner spike was not fired, since the much-reduced e.p.s.p. (marked by an arrow) was well below threshold.

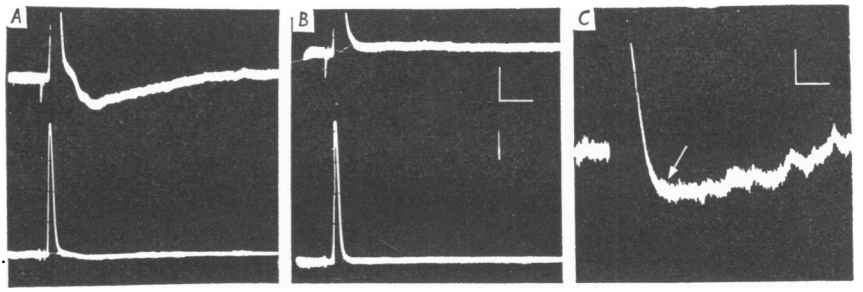

Fig. 4. Inhibitory potentials in intoxicated fish. *A* and *B*. The upper and lower traces are high and low gain records respectively of the intracellularly recorded response of the Mauthner cell to an antidromic stimulus. The inhibitory hyperpolarization in *A* (following the Mauthner cell spike) is absent in *B*, which shows superimposed traces of antidromic responses repeated at 10/sec (see text).

C. The arrow indicates the hyperpolarization produced in the Mauthner cell by excitation of the *opposite* VIIIth nerve. The initial upward deflexion is the stimulus artifact.

Calibrations: vertical: 1 mV and 10 mV (*A* and *B*) and 0·2 mV (*C*); horizontal: 5 msec (*A* and *B*) and 2 msec (*C*).

Similar records to those shown in Fig. 3 were obtained on all occasions when the Mauthner cell was investigated in behaviourally abnormal, intoxicated, fish. These results, both of inhibition (the collateral *and* the contralateral VIIIth nerve) and of excitation (orthodromic and antidromic) were completely characteristic of normal goldfish. It appears therefore that the apparent 'convulsions' were occurring without any detectable effect on at least these particular inhibitory (and excitatory) systems acting on the Mauthner cell.

All fish which had been injected with a lethal dose of tetanus toxin eventually died with a flaccid paralysis, and many of these died without an intervening period of 'convulsive' behaviour as described above. Mauthner cells were also investigated in a number of fish which were dying in the flaccid condition. Again, in these fish, the inhibition and

excitation of the Mauthner cell was completely normal. Sometimes the inhibitory conductance increase of the Mauthner cell is associated with a hyperpolarization of up to about 2 mV (Furukawa & Furshpan, 1963). Fig. 4 shows records obtained from a Mauthner cell in a flaccidly paralysed fish, and demonstrates that this hyperpolarizing inhibitory post-synaptic potential could still be present. In records *A* and *B*, an antidromically excited spike is shown at low gain (lower trace) and at high gain (upper trace). The hyperpolarization which occurred during the period when the collateral inhibition was shown to act can be clearly seen following the spike in the upper record of Fig. 4*A*. In 4*B* the antidromic stimulation was repeated at 10 times a second, when the collateral inhibition fatigues out; the hyperpolarization (and the inhibition) was now absent. Fig. 4*C* shows a hyperpolarization (indicated by the arrow) following the excitation of the contralateral VIIIth nerve, which caused the type of inhibition shown in Fig. 3*B* (this inhibitory potential is preceded by a large stimulus artifact). Unlike the collateral inhibition, the crossed VIIIth nerve inhibition does not fatigue appreciably even at stimulation frequencies of 10/sec or more.

Direct application of toxin to the brain

Since the above experiments had revealed no abnormalities in the various excitatory and inhibitory systems acting on the Mauthner cell, it was possible that the fish were dying of a peripheral paralytic action of the toxin before any central action had had time to occur. In order to be sure that a lack of central effects was not due merely to an inability of the toxin to enter the central nervous system, the toxin was injected directly over the brain through a hole drilled in the skull. In order to give the toxin as much time as possible to act on the central nervous system, the fish were kept 'alive' after they had become totally paralysed, by continuously perfusing them with aerated water into the mouth and out through the gills. Under these circumstances, in some fish the heart would continue to beat for several days (although in many cases it would stop after a few hours). In this way Mauthner cells were investigated in fish which had been completely paralysed for up to 3 days.

However, even in these fish no abnormalities in either inhibition or excitation of the Mauthner cells were detected; collateral inhibition and contralateral VIIIth nerve inhibition were found to be acting entirely normally. Fig. 5 illustrates that the principal mechanism underlying the inhibition was also still working normally. In this experiment large ($1\frac{1}{2}\ \mu$ tip diameter) KCl-filled micro-electrodes were used, so that there was a marked diffusion of chloride into the cell. The mechanism of chemically transmitted, post-synaptic inhibition of the Mauthner cell, as in so many neurones, involves a striking increase in the chloride conductance of the

membrane, and in these experimental circumstances the activation of the inhibitory synapses results in depolarization of the cell (Furukawa & Furshpan, 1963). If this depolarization is large enough, it can act as an e.p.s.p., and itself initiate a spike, i.e. the inhibition can be converted into an excitation; this phenomenon is entirely dependent on the attainment of a large enough concentration of chloride inside the cell, and then will occur in normal Mauthner cells, as in normal motoneurones of the cat

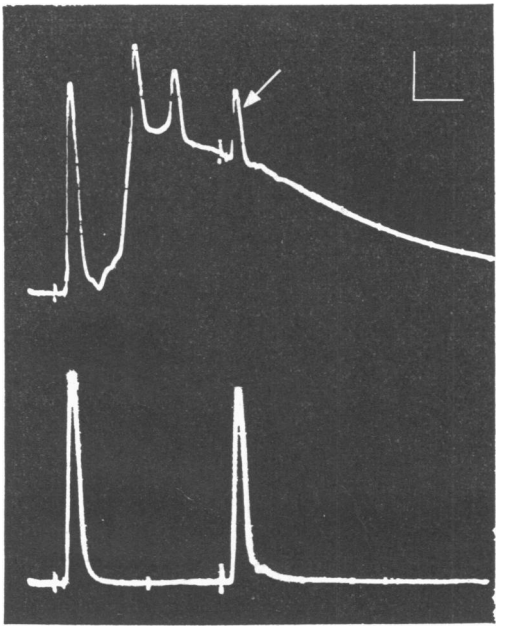

Fig. 5. The collateral 'inhibition' during Cl diffusion into the Mauthner cell in intoxicated fish. The effect of the increased intracellular concentration of Cl is to convert the inhibition into an excitation. The cell was excited antidromically by a pair of stimuli each of which fired a spike. The second spike is indicated by the arrow in the upper record. The two intervening spikes in the upper record were caused by the enormous depolarization attributable to activation of the collateral inhibitory systems. The lower record consists of superimposed traces of the response when the pair of stimuli was delivered at 10/sec (cf. Fig. 3 and text).
Calibrations: vertical: 5 mV; horizontal: 2 msec.

spinal cord (Coombs, Eccles & Fatt, 1955). In the upper record of Fig. 5 the collateral inhibition which follows the antidromic spike was converted into an excitatory depolarization in this way, and in this instance itself fired two spikes. The spike marked by an arrow was produced by a second antidromic stimulus. In the lower record the pair of antidromic stimuli was repeated at 10 times a second, at which frequency the collateral inhibition fatigued out, the chloride conductance change was no longer

produced, and hence the depolarization was absent. The two antidromic spikes now had their usual characteristics (cf. Fig. 3*A*).

Peripheral effects of tetanus toxin

Fish were injected with 200 LD_{50} (mouse) of tetanus toxin into the pectoral fin muscle on one side, and the same amount of toxin, mixed with a neutralizing dose of antitoxin, was injected into the corresponding muscle of the other side. This dose of toxin, at 14° C, is sublethal. The fish

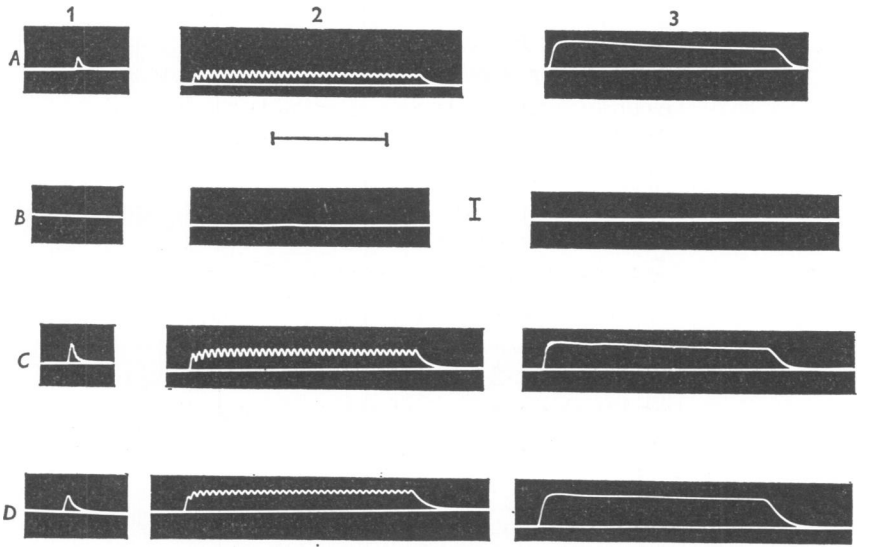

Fig. 6. Muscle tension records from tetanus toxin-injected fish. Records from right (*B* and *D*) and left (*A* and *C*) fins of same fish. The right fin muscle was injected with tetanus toxin and the left with tetanus toxin mixed with a neutralizing dose of antitoxin. *A* and *B* show responses to stimulus of the nerves; *C* and *D* show responses to direct stimulation of the muscle. Left-hand columns, single maximum twitch. Middle columns, tetanic stimulation at 16/sec. Right-hand columns, tetanic stimulation at 60/sec.

Calibrations: vertical: 20 g; horizontal: 1 sec.

were killed at various times after the injection when some abnormality was visible in the movement of the fin, and the fin muscle and its nerve then dissected out from each side. The preparation was set up so that the nerve or the muscle could be stimulated electrically, and the mechanical response of the muscle recorded.

At about 4 days after the local injection of toxin the mechanical response to nerve stimulation was always weaker on the toxin-injected side; by 6 days the muscle barely responded, or responded not at all, to stimulation of its nerve (Fig. 6*A* and *B*). Paralysed fin muscles, left *in vivo*, did not

recover during periods of observation of which the longest was 30 days. The response of the muscle to direct electrical stimulation was tested immediately after recording the response to stimulation of the nerve. All the muscle still responded to direct electrical stimulation (Fig. 6*C* and *D*). Thus the weakened or absent response to stimulation of the nerve in intoxicated muscles was not due to a failure of the muscular response itself. However, in preparations which had been paralysed for 3 or more days before their isolation from the animal, the responses to direct electrical stimulation were always less than those of the control muscles.

Since the toxin-paralysed muscles responded to direct stimulation, the paralysis could be due either to a block of neuromuscular transmission or to a block in nerve conduction. The second possibility was tested by recording the compound action potential from the nerve supplying a paralysed fin, and comparing it with the record from the opposite, control fin. No marked differences were found in the size or shape of the compound action potentials. The stimulus parameters used to obtain this *maximum* compound action potential caused the *maximum* mechanical response in the non-paralysed muscle. It seems therefore that either (1) the impulse conduction in the pre-terminal portions of the motor nerve is affected by the tetanus toxin, and/or (2) there is prevention of acetylcholine release from the endings and/or (3) the response of the post-synaptic region of the muscle fibre to the neural transmitter (acetylcholine), is blocked.

DISCUSSION

The inhibitory systems acting on the Mauthner cells of goldfish appear to be insensitive to the action of tetanus toxin, which produces such dramatic effects in mammals. However, the toxicity of tetanus toxin to goldfish is comparable to its toxicity to mice. Thus at 28° C a 60 g fish will die within 5 days from only 20 times the weight of toxin which would take the same time to kill a 20 g mouse (at 38° C). This contrasts markedly with the frog, which (at 28° C) is at least 1000 times less sensitive to tetanus toxin than is the mouse.

The goldfish apparently dies from a peripheral, paralytic action of tetanus toxin, which seems superficially comparable with the action of botulinum toxin. It has been found in preliminary experiments (J. H. Mellanby, unpublished observations) that botulinum toxin injected into the fin of goldfish produces symptoms indistinguishable from those produced by tetanus toxin. Twenty years ago the two toxins were shown to have similar effects on another cholinergic system, in the rabbit. Thus Ambache *et al.* (1948*a*) (see also Mellanby, Pope & Ambache, 1968) showed that both toxins, when injected into the anterior chamber of the eye, pre-

vented the response of the *sphincter pupillae* muscle to stimulation of the oculomotor nerve (which is preganglionic).

There is other evidence in the literature suggesting that under some circumstances it is possible to produce a flaccid instead of a spastic paralysis with tetanus toxin. Thus Davies & Wright (1955) found that when mice were injected intravenously with a large dose of tetanus toxoid (330 Lf) at the same time as they were given tetanus toxin (36 LD_{50}) they could be protected from the tetanus toxin. If the dose of toxoid was insufficient to protect the mice completely, then there were no convulsions or muscular spasms, and instead the muscles appeared to be flaccid. Miyasaki *et al.* (1967) reported that if an intramuscular injection of tetanus toxin in rabbits is followed 2 hr later by intravenous injection of antitoxin, a predominantly flaccid paralysis develops. Similar flaccidity was also produced when the toxin was injected into the epineurium of the ischiadicus nerve. These authors concluded that tetanus toxin has two pharmacological actions, one central and one peripheral. A similar conclusion was reached by Prabhu, Oester & Karczmar (1962). Prabhu & Oester (1962) suggested that in rabbits, intramuscularly injected tetanus toxin has a dual action – a central one occurring first, followed after some days by a local neuromuscular blocking action on the injected muscle. Muchnik & Rubinstein (1967) found that with the rat isolated phrenic–hemidiaphragm preparation tetanus toxin blocked the mechanical response of the muscle to stimulation of its nerve $\frac{1}{2}$–3 hr after it was added to the bath. Surprisingly, since they did not record the response of the muscle to direct stimulation, they concluded that the toxin interfered directly with the contractile mechanism in the muscle.

Our conclusion from the present experiments on goldfish is that tetanus toxin has a peripheral site of action, probably at the neuromuscular junction, and that goldfish die as a result of the paralysis caused by this action. We have no direct evidence that in the goldfish the toxin has any central action at all; the occasional erratic swimming seen in intoxicated fish could be attributable to the disorganization of movement, and therefore of sensory feed-back, caused by the peripheral action of the toxin. Although we have some preliminary observations suggesting a possible direct action of the toxin on the muscle itself it seems that the major part of the effects can be attributed to blocking of transmission between nerve and muscle. In preliminary experiments the miniature end-plate potentials, which are readily recorded in normal fin muscles, appear to be absent in paralysed ones (J. H. Mellanby and P. Thompson, unpublished). Further experiments are in progress to check whether the effect of the toxin is largely presynaptic, as in the case of botulinum toxin, or whether there is an associated reduction in post-synaptic sensitivity to acetylcholine. The

absence of any central action of the toxin is surprising but we have not excluded the possibility that the peripheral system is so highly sensitive to tetanus toxin in the goldfish that there is not enough time for a central action to be manifested. Nevertheless, even when the toxin was applied directly over the brain and the animal was kept alive for as long as 72 hr after the total peripheral paralysis had occurred, we were still unable to detect any interference with excitation or inhibition acting on the Mauthner cells.

The apparent differences between the susceptibility to tetanus toxin of inhibition acting on the Mauthner cell and of that acting on spinal neurones in the cat are surprising. Both inhibitions are blocked by strychnine; both are mimicked by glycine, whose action is also prevented by strychnine. In a recent report it was suggested that strychnine could be acting presynaptically on the systems inhibiting the Mauthner cells, or possibly that glycine may not be eligible as the inhibitory transmitter at the Mauthner cell (Roper *et al.* 1969; Roper & Diamond, 1970). The evidence for the motoneurone has been interpreted very strongly in favour of glycine as the inhibitory transmitter (Curtis & Johnston, 1970). Our feeling is that at present we cannot distinguish between the possibilities (1) that there are subtle but important differences between the fish and the mammalian inhibitory systems, and (2) that the apparent differences are dependent on the differences of the experimental conditions in which similar techniques were used. Certainly, in the present experiments the inhibition of the Mauthner cells in tetanus-intoxicated fish was readily abolished by strychnine. Until comparative data are available for different neurones in different parts of the brain of different animal types, it may be important not to generalize from results obtained from relatively few kinds of neuronal inhibitory systems.

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REFERENCES

AMBACHE, N. (1949). The peripheral action of *Cl. botulinum* toxin. *J. Physiol.* **108**, 127–141.

AMBACHE, N., MORGAN, R. S. & WRIGHT, G. P. (1948*a*). The action of tetanus toxin on the rabbit's iris. *J. Physiol.* **107**, 45–53.

AMBACHE, N., MORGAN, R. S. & WRIGHT, G. P. (1948*b*). The action of tetanus toxin on the acetylcholine and cholinesterase contents of the rabbit's iris. *Br. J. exp. Path.* **29**, 408–418.

BROOKS, V. B. (1956). An intracellular study of the action of repetitive nerve volleys and of botulinum toxin on miniature end-plate potentials. *J. Physiol.* **134**, 264–277.

BROOKS, V. B., CURTIS, D. R. & ECCLES, J. C. (1957). The action of tetanus toxin on the inhibition of motoneurones. *J. Physiol.* **135**, 655–672.

COOMBS, J. S., ECCLES, J. C. & FATT, P. (1955). The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory post-synaptic potential. *J. Physiol.* **130**, 326–373.

COWLES, R. B. & NELSON, N. B. (1947). Studies on thermal sedation in suppression of the symptoms of tetanus toxin. *Proc. Soc. exp. Biol. Med.* **64**, 220–224.

CURTIS, D. R. & DE GROAT, W. C. (1968). Tetanus toxin and spinal inhibition. *Brain Res.* **10**, 208.

CURTIS, D. R. & JOHNSTON, G. A. R. (1970). Strychnine, glycine and vertebrate post-synaptic inhibition. *Nature, Lond.* **225**, 1258–1259.

DAVIES, J. R., MORGAN, R. S. & WRIGHT, E. A. (1955). The susceptibility of pigeons to tetanus toxin. *J. Path. Bact.* **69**, 295–298.

DAVIES, J. R. & WRIGHT, E. A. (1955). The specific precocious protective action of tetanus toxoid. *Br. J. exp. Path.* **36**, 487–493.

DIAMOND, J. (1968). The activation and distribution of GABA and L-glutamate receptors on goldfish Mauthner neurones. An analysis of dendritic remote inhibition. *J. Physiol.* **194**, 669–723.

DIAMOND, J. & MELLANBY, J. H. (1970). The effect of tetanus toxin in the goldfish. *J. Physiol.* **210**, 186–187 *P.*

ECCLES, J. C. (1964). *The Physiology of Synapses*, p. 191. Berlin: Springer-Verlag.

FURSHPAN, E. J. & FURUKAWA, T. (1962). Intracellular and extracellular responses of the several regions of the Mauthner cell of the goldfish. *J. Neurophysiol.* **25**, 732–771.

FURUKAWA, T. (1966). Synaptic interaction at the Mauthner cell of goldfish. *Prog. Brain Res.* **21A**, 46–70.

FURUKAWA, T. & FURSHPAN, E. J. (1963). Two inhibitory mechanisms in the Mauthner cells of goldfish. *J. Neurophysiol.* **26**, 140–176.

KAESER, H. E. & SANER, A. (1969). Tetanus toxin, a neuromuscular blocking agent. *Nature, Lond.* **223**, 842.

MELLANBY, J. H., POPE, D. L. & AMBACHE, N. (1968). The effect of the treatment of crude tetanus toxin with ganglioside-cerebroside complex on sphincter paralysis in the rabbit eye. *J. gen. Microbiol.* **50**, 479–486.

MIYASAKI, S., OKADA, K., MUTO, S., ITOKAZU, T., MATSUI, M., EBISAWA, I., KAGABE, K. & KIMURO, T. (1967). On the mode of action of tetanus toxin in rabbit. I. Distribution of tetanus toxin *in vivo* and development of paralytic signs under some conditions. *Japan J. exp. Med.* **37**, 217–225.

MUCHNIK, S. & RUBINSTEIN, E. H. (1967). Mechanism of the local tetanus induced by intramuscular tetanus toxin. *Acta physiol. latinoam.* **17**, 166–174.

PRABHU, V. G. & OESTER, Y. T. (1962). Electromyographic changes in skeletal muscle due to tetanus toxin. *J. Pharmac. exp. Ther.* **138**, 241–248.

PRABHU, V. G., OESTER, Y. T. & KARCZMAR, A. G. (1962). Some observations on the actions of certain pharmacological agents on the fibrillation potentials and on the sensitivity of the skeletal muscle of rabbit in advanced local tetanus. *Int. J. Neuropharmac.* **1**, 371–379.

ROPER, S. & DIAMOND, J. (1970). Strychnine antagonism and glycine: a reply. *Nature, Lond.* **225**, 1259.

ROPER, S., DIAMOND, J. & YASARGIL, G. M. (1969). Does strychnine block inhibition post-synaptically? *Nature, Lond.* **223**, 1168–1169.

ROWSON, K. E. K. (1961). The action of tetanus toxin in frogs. *J. gen. Microbiol.* **25**, 315–329.

VAN HEYNINGEN, W. E. & MELLANBY, J. H. (1970). In *Microbial Toxins*, ed. AJL, S. J. New York: Academic Press Inc. (in the Press).