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EFFECT OF TETANUS TOXIN ON THE EXCITATORY AND THE INHIBITORY POST-SYNAPTIC POTENTIALS IN THE CAT MOTONEURONE

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SUMMARY

1. Tetanus toxin (100 mouse minimal lethal doses per kilogram) was injected into the medial gastrocnemius muscle of the cat. At various times thereafter, homonymous and heteronymous group Ia excitatory post-synaptic potentials (e.p.s.p.s), disynaptic reciprocal Ia inhibitory post-synaptic potentials (i.p.s.p.s) and post-synaptic potentials (p.s.p.s) produced by sural nerve stimulation were recorded in the medial gastrocnemius motoneurones. The duration of the after-hyperpolarization, the input resistance and the axonal conduction velocity of motoneurones were also measured.

2. Homonymous Ia e.p.s.p.s remained normal until 72 h after toxin injection. However, 5 days after toxin injection, the amplitudes of Ia e.p.s.p.s were significantly smaller than those in control animals ($1{\cdot}5 \pm 1{\cdot}0$ mV *versus* $5{\cdot}6 \pm 2{\cdot}7$ mV; *t* test, $P < 0{\cdot}001$).

3. Heteronymous Ia e.p.s.p.s produced by stimulation of the lateral gastrocnemius–soleus nerve 5 days after toxin injection were also significantly smaller than those in control animals ($0{\cdot}6 \pm 0{\cdot}6$ mV *versus* $2{\cdot}5 \pm 1{\cdot}5$ mV; $P < 0{\cdot}001$). However, these heteronymous Ia e.p.s.p.s remained normal when the lateral gastrocnemius–soleus nerve was ligated and sectioned at the entry to those muscles just before the toxin injection.

4. The ascending volleys, which are supposed to represent mainly the action potentials of the dorsal spinocerebellar tract and to be elicited monosynaptically by collaterals of group I afferents, were essentially the same in the left tetanic and right control sides up to 5 days after toxin injection.

5. Ia i.p.s.p.s and the hyperpolarizing component of sural p.s.p.s could not be produced or were very small in motoneurones sampled later than 30 h after toxin injection.

6. The duration of the after-hyperpolarization and the input resistance of motoneurones remained normal. Axonal conduction velocity of motoneurones measured 5 days after toxin injection was $89{\cdot}4 \pm 12{\cdot}7$ m/s, and was significantly slower than that of control motoneurones ($94{\cdot}1 \pm 15{\cdot}4$ m/s) ($P < 0{\cdot}005$). Differences in the

* The laboratory where the present experiments were carried out.

amplitude of group I incoming volleys between tetanic leg and contralateral control leg were not observed.

7. These results suggest that tetanus toxin blocks excitatory synapses in the central nervous system as well as inhibitory synapses.

INTRODUCTION

Sherrington (1905) found that the inhibition of extensor reflexes was replaced by facilitation after the application of tetanus toxin or strychnine. He suggested that this transformation was attributable to the conversion of the inhibitory process into an excitatory one in the spinal cord. Later, Brooks, Curtis & Eccles (1957) showed that tetanus toxin blocked various kinds of spinal inhibitory reflex action without any effect on the monosynaptic reflex. Since then it has been shown that many kinds of inhibitory synapses in the central nervous system (C.N.S.) are blocked by tetanus toxin (Brooks & Asanuma, 1962; Curtis & De Groat, 1968; Curtis, Felix, Game & McCulloch, 1973; Davies & Tongroach, 1979) while some excitatory synapses in the spinal cord and the substantia nigra are not affected (Brooks *et al.* 1957; Geinisman, D'yakonova & Kryzhanovsky, 1967; Curtis & De Groat, 1968; Kryzhanovsky, Kurchavyi & Sheikhon, 1973; Takano, 1976; Davies & Tongroach, 1979). Therefore, it has been generally considered that tetanus toxin does not act directly on excitatory synapses in the C.N.S. (see Mellanby & Green, 1981).

On the other hand some cholinergic synapses in the mammalian peripheral nervous system, which are excitatory in nature, have been reported to be blocked by tetanus toxin (Ambache, Morgan & Wright, 1948; Duchen & Tonge, 1973; Kryzhanovksy, 1973; Takano, 1976; Kretzschmar, Kirchner & Takano, 1980). Sverdlov (1960) and Terhaar, Tiebert, Kirchner & Takano (1977) reported that the monosynaptic reflex recorded from the ventral root eventually decreased in amplitude when local tetanus was produced in cats and when the dose of tetanus toxin injected into a muscle was rather small and the animals survived for a long period. It was also noticed by Mikhailov & Shvarts (1969) that only subthreshold Ia e.p.s.p.s could be observed in most motoneurones on the ninth to sixteenth day after tetanus poisoning.

These observations led us to investigate the effect of low doses of tetanus toxin on certain excitatory and inhibitory post-synaptic potentials produced in motoneurones and some of the electrophysiological properties of the motoneurone. The results suggest that an excitatory synapse in the C.N.S., namely the group Ia synapse on the spinal motoneurones, may also be blocked by a small amount of tetanus toxin injected into a leg muscle. This effect was delayed by about 4 days in comparison with the well-known effect on inhibitory synapses. Preliminary reports of some of this material have appeared elsewhere (Kanda & Takano, 1979; Kanda, 1980*a*, *b*; Takano & Kanda, 1980).

METHODS

Preparation

Experiments were performed on thirty-nine adult cats. The non-purified toxin (T7, Behringwerke) was dissolved in Ringer solution just before injection. This solution had the toxicity of 1000 mouse minimal lethal doses (m.l.d.) per millilitre. In twenty-two cats, tetanus toxin (100 mouse m.l.d. per kilogram of body weight) was injected into the central portion of the muscle belly using multiple

penetration, ensuring that the needle always remained within the left medial gastrocnemius (m.g.) muscle. The toxin injection was done under ketamine anaesthesia. Symptoms of local tetanus appeared in the injected leg 24–36 h after toxin injection; the contralateral leg did not show any neurological signs of tetanus throughout the observation period of the present experiments. In six other cats, just before the toxin injection (the same amount as for the previous group) into the left m.g. muscle, the left lateral gastrocnemius–soleus (l.g.s.) nerve was ligated and sectioned peripherally. In five other cats the l.g.s. nerve was sectioned but toxin was not injected. These procedures were performed under light sodium pentobarbitone anaesthesia. The remaining six cats were intact controls.

The acute experiments on tetanic cats with intact l.g.s. nerve were performed 1–5 days after toxin injection, but those on cats with l.g.s. nerve sectioned only 5 days after the operation. Cats were surgically prepared under sodium pentobarbitone anaesthesia (initial dose: 35–40 mg/kg I.P.). Subsequently, intermittent small intravenous doses of the same anaesthetic were used to maintain a level of anaesthesia throughout the experiment. Blood pressure and body temperature were monitored continuously. The animals were mounted in a metal frame immobilizing the lumbosacral spine and hind legs. The nerves to the left m.g. and l.g.s. muscles were dissected free from the surrounding tissue and placed on bipolar platinum hooks for stimulation. In five cats the right m.g. and l.g.s. nerves were also dissected and placed on the stimulating electrodes. The nerves to the left tibialis anterior and extensor digitorum longus (t.a.–e.d.l.) muscles, and to the skin (i.e. sural nerve: Sur) were cut and placed on bipolar stimulating electrodes. The cathode was the proximal element of each electrode pair. The lumbosacral spinal cord was exposed through a laminectomy (L_4–L_7). In five cats another laminectomy (T_{10}–L_1) was performed in order to record the ascending volley. Dorsal and ventral roots of the lumbar spinal cord were intact.

Recordings

M.g. motoneurones, identified initially by antidromic invasion (Fig. 1 *A*, *F*, *K*), were penetrated with glass micropipettes filled with 2 M-potassium citrate. In order to examine homonymous, monosynaptic group Ia excitatory post-synaptic potentials (Ia e.p.s.p.s) from m.g. afferents, the intact m.g. nerve was stimulated at various strengths. Antidromic invasion of the m.g. motoneurone was blocked by passing long hyperpolarizing pulses, leaving the Ia e.p.s.p. superimposed on an M spike (Fig. 1 *C*, *H*, *M*). The heteronymous Ia e.p.s.p.s were recorded simply by stimulation of the l.g.s. nerve with pulses slightly supramaximal for group I afferents. The disynaptic Ia inhibitory post-synaptic potentials (Ia i.p.s.p.s) were produced in m.g. motoneurones by stimulation of the t.a.–e.d.l. nerve at various strengths, mostly just supramaximal for group I afferents (Fig. 1 *D*, *I*, *N*). We did not use any facilitation techniques such as stimulation of the ipsilateral dorsal quadrant of the spinal cord (see Burke, Rymer & Walsh, 1976). The strength of sural stimulation used in this experiment was relatively weak, usually around five times threshold for this nerve. The duration of the after-hyperpolarization was measured using a brief depolarizing current through the micro-electrode to discharge the motoneurone (Fig. 1 *B*, *G*, *L*). The conduction velocity of the motor axon was calculated from the latency of the antidromic spike and the conduction distance, which was measured at the end of the experiment. The input resistance of motoneurones was also measured by the spike height method (Nelson & Frank, 1967). Data were accepted from motoneurones with resting potentials greater than −50 mV.

Afferent volleys were recorded with a platinum ball electrode near the L_7 dorsal root entry. When the group I afferent volley elicited by stimulation of left and right m.g. nerves was examined, the ball electrode was placed on the mid line of the dorsal column of the spinal cord at L_7. Ascending volleys in the spinal tract fibres were recorded from the dissected dorsal quadrants by bipolar platinum hooks. It was, however, very difficult to compare the amplitude of volleys of the two sides of the dorsal quadrant because the surgical dissection and the recording conditions were not exactly identical. Therefore, we also recorded the ascending volleys by the ball electrode placed on the mid line of the dorsal column surface at the T_{10-12} level. The dorsal column was removed over a length of about 5 mm at the L_1 level. The ascending volleys were averaged fifty to a hundred times.

RESULTS

Group Ia e.p.s.p.s

It is known that the amplitude of maximum homonymous Ia e.p.s.p.s. in cat m.g. motoneurones depends on the motor unit type (Burke *et al.* 1976), and that the distribution of each type of motor unit is not uniform but skewed (Burke, Strick, Kanda, Kim & Walmsley, 1977). We did not identify the motor unit type of each motoneurone because of technical difficulties in tetanic animals, especially in the late

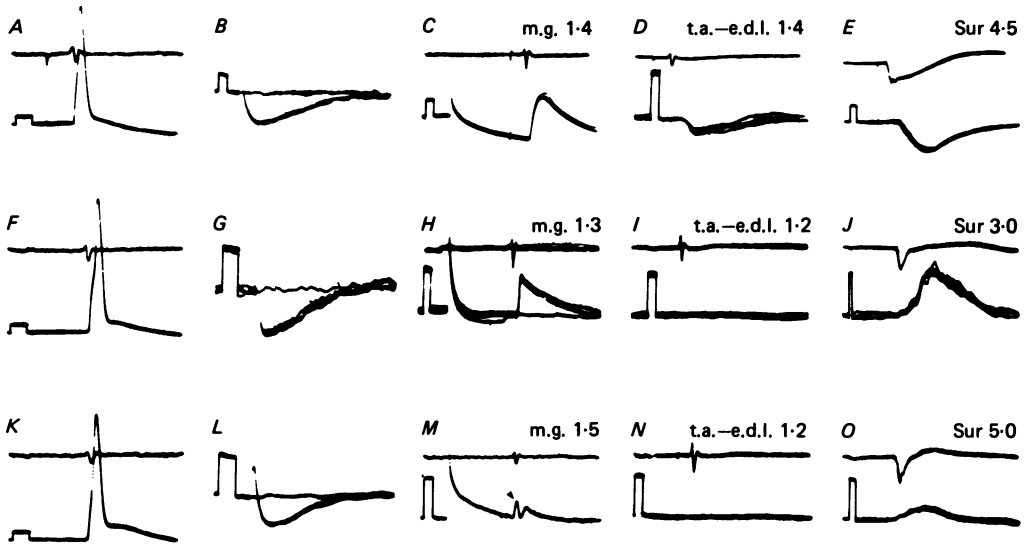

Fig. 1. Examples of intracellular recordings from m.g. motoneurones sampled in control cats (*A–E*) and cats with local tetanus (*F–J*, 2 days after toxin injection; *K–O*, 5 days after). *A*, *F* and *K*: antidromic spikes (lower traces) and cord dorsum potentials (upper traces). *B*, *G* and *L*: after-hyperpolarization following a spike produced by injecting a short depolarizing current throuh the micro-electrode. *C*, *D*, *E*, *H*, *I*, *J*, *M*, *N* and *O*: post-synaptic potentials were generated by single-shock stimulation of the various peripheral nerves at strengths indicated (in multiples of threshold) above each record. Homonymous group Ia e.p.s.p.s were elicited during passage of a hyperpolarizing current into the motoneurone to prevent spike generation. Note that in record *M* only a very small e.p.s.p. following the M spike (arrow) was recorded 5 days after toxin injection. Calibration pulses: 5 mV and 1 ms in *A*, *C*, *D*, *F*, *H*, *I*, *K*, *M* and *N*; 5 mV and 10 ms in *B*, *G* and *L*; 5 mV and 2 ms in *E*, *J* and *O*.

period of intoxication. However, we tried to sample motoneurones at various sites within the m.g. motor nucleus of each animal. Data from the motoneurones of tetanic cats were compared with those of control cats. The mean homonymous Ia e.p.s.p. amplitude in control animals was $5{\cdot}6 \pm 2{\cdot}7$ (S.D.) mV ($n = 78$; Fig. 2*A*). The average amplitude ($5{\cdot}2 \pm 2{\cdot}1$ mV) of Ia e.p.s.p.s ($n = 42$) sampled between 30 and 48 h after toxin injection was not significantly different (t test, $P > 0{\cdot}1$) from the control value (Fig. 2*B*). The amplitude ($4{\cdot}9 \pm 2{\cdot}3$ mV) of the Ia e.p.s.p.s elicited in motoneurones ($n = 40$) between 55 and 72 h after toxin injection was still essentially the same ($P > 0{\cdot}1$) as the control value (Fig. 2*C*). However, after 5 days the maximum

homonymous Ia e.p.s.p.s elicited in m.g. motoneurones decreased in amplitude considerably. The mean value was $1{\cdot}5 \pm 1{\cdot}0$ mV ($n = 101$), which was significantly smaller than that in control animals ($P < 0{\cdot}001$; Fig. 2*D*).

The heteronymous Ia e.p.s.p.s elicited in m.g. motoneurones also decreased in amplitude 5 days after toxin injection. The mean value was $0{\cdot}6 \pm 0{\cdot}6$ mV ($n = 42$), which was significantly smaller than that in control animals ($2{\cdot}5 \pm 1{\cdot}5$ mV; $n = 63$; $P < 0{\cdot}001$) (Fig. 3).

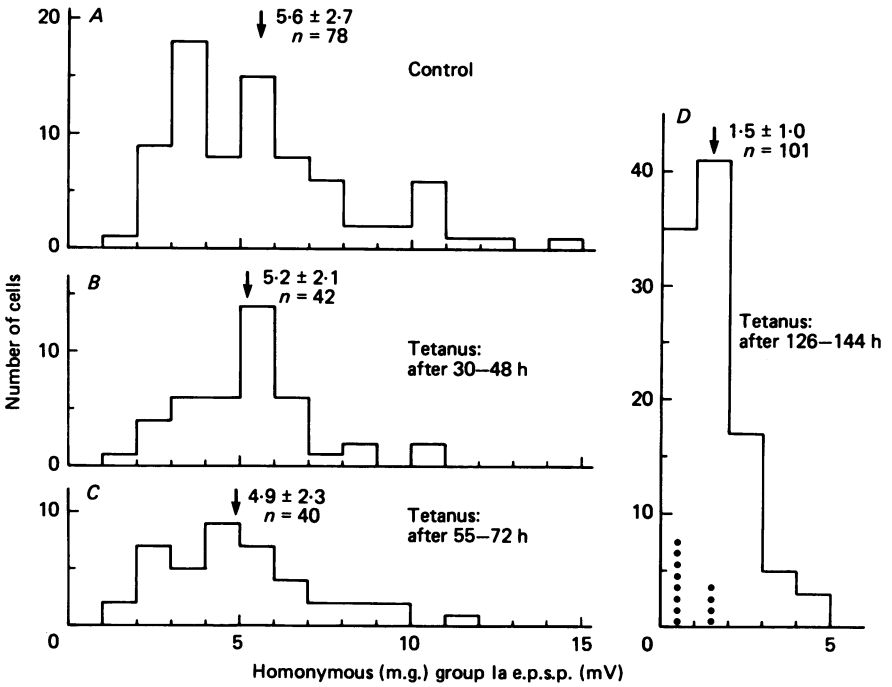

Fig. 2. Histograms showing the distributions of the amplitudes of maximum homonymous group Ia e.p.s.p.s elicited in m.g. motoneurones. Arrows indicate the mean values in each histogram. The mean values of the amplitude of the Ia e.p.s.p.s recorded from motoneurones 30–48 h and 55–72 h after toxin injection are not statistically different from those in control animals, while those of motoneurones recorded after 126–144 h are significantly smaller. Dots designate the data from cat T45 in Table 1.

Effect of l.g.s. nerve section

As shown in Figs. 2 and 3, both homonymous and heteronymous Ia e.p.s.p.s were equally decreased in amplitude after 5 days, although the toxin was carefully injected only into the left m.g. muscle. We examined the effect of left l.g.s. nerve section, which might prevent this nerve from transporting tetanus toxin retrogradely. In this case the data sampled from tetanic cats with l.g.s. nerve section were compared with those from cats in which the l.g.s. nerve had been sectioned but no toxin injected. Intracellular recording from m.g. motoneurones was performed 5 days after the operation (see Methods). It has been reported that peripheral nerve section itself does not produce any detectable changes in Ia e.p.s.p.s in motoneurones up to 6 days after

the operation (Eccles, Krnjević & Miledi, 1959). Fig. 4*D* shows the maximum amplitudes of homonymous and heteronymous Ia e.p.s.p.s elicited in m.g. motoneurones of both control (filled circles, $n = 48$) and tetanic (open circles, $n = 49$) cats. As shown in Fig. 4*D*, the Ia e.p.s.p.s evoked by stimulation of the m.g. nerve in tetanic cats were significantly decreased in amplitude in comparison with those in

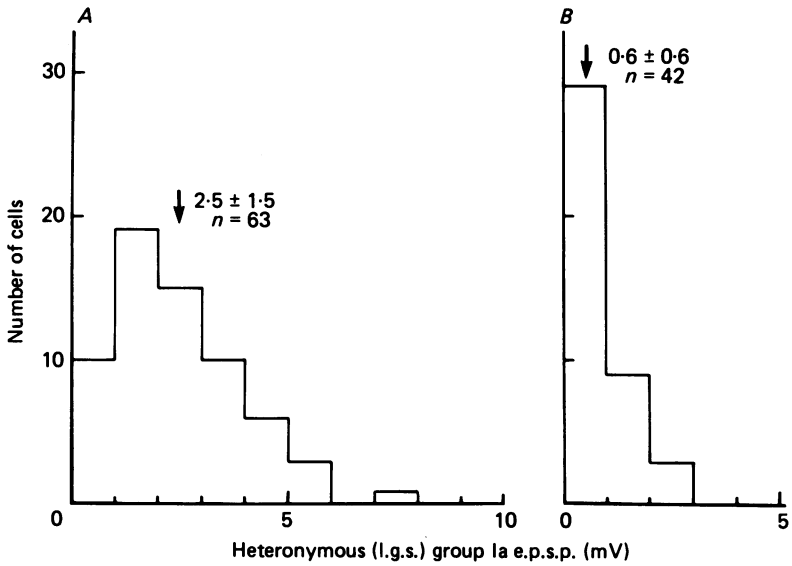

Fig. 3. Histograms showing the distributions of the amplitudes of maximum heteronymous group Ia e.p.s.p.s produced by stimulation of the l.g.s. nerve. Arrows indicate the mean values in each histogram. The mean value of the amplitude of the Ia e.p.s.p.s recorded from motoneurones 5 days after toxin injection (*B*) is significantly smaller than that in control cats (*A*).

control cats ($2{\cdot}0 \pm 1{\cdot}2$ mV *versus* $5{\cdot}9 \pm 2{\cdot}7$ mV; $P < 0{\cdot}001$). On the other hand, those evoked by stimulation of the l.g.s. nerve, which had been sectioned previously, remained unchanged ($3{\cdot}3 \pm 1{\cdot}6$ mV *versus* $2{\cdot}8 \pm 1{\cdot}5$ mV).

Ascending volley elicited by stimulation of group I afferents

We also examined the effect of tetanus toxin on the synaptic transmission between group I afferents and ascending tract neurones. The ascending volleys in response to stimulation of the left, tetanic m.g. nerves and right, intact m.g. nerves were recorded. These volleys are supposed to represent mainly the action potentials of the dorsal spinocerebellar tract and to be elicited monosynaptically by collaterals of group I afferents (Lloyd & McIntyre, 1950; Laporte, Lundberg & Oscarson, 1956*a*, *b*). We chose the contralateral intact side, which showed no neurological sign of tetanus, as control in this case (see Methods). As shown in Fig. 5 and Table 1, the ascending volleys appeared to be essentially the same in the left and right sides up to 5 days after toxin injection.

T.a.–e.d.l. disynaptic group Ia i.p.s.p.s

Under our experimental conditions, the amplitudes of i.p.s.p.s produced in m.g. motoneurones by stimulation of group I afferents from t.a.–e.d.l. nerve were rather diverse in control cats (Fig. 6*A*). The average amplitude for thirty-four Ia i.p.s.p.s in control animals was 0·8 ± 0·8 mV. This Ia i.p.s.p. was hardly ever observed even in the early period of tetanus intoxication (Fig. 1*I*, *N*; Fig. 6*B*). The amplitude of

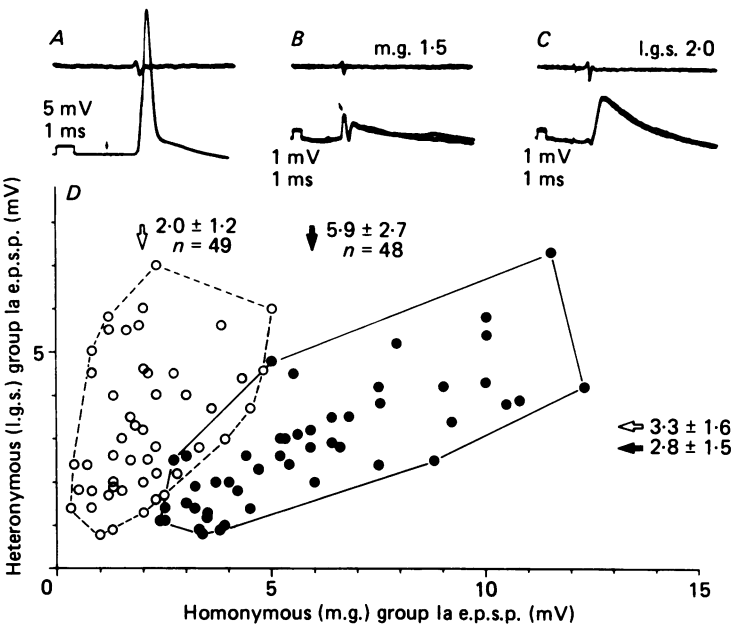

Fig. 4. Relation between the maximum amplitudes of homonymous (m.g.) and heteronymous (l.g.s.) Ia e.p.s.p.s in m.g. motoneurones recorded from control and tetanic cats in which the l.g.s. nerve had been sectioned. Examples of intracellular recordings from a m.g. motoneurone (lower traces) and cord dorsum potentials (upper traces) obtained in a cat with local tetanus (*A–C*). *A*, antidromic spike potential; *B*, maximum homonymous Ia e.p.s.p.; hyperpolarizing current was passed to prevent spike generation. An arrow indicates the M spike. *C*, maximum heteronymous Ia e.p.s.p. produced by stimulation of l.g.s. nerve. Stimulated nerves and stimulus strengths (in multiples of threshold) are indicated above records in *B* and *C*. *D*, maximum amplitudes of homonymous (on the abscissa) and heteronymous (on the ordinate) Ia e.p.s.p.s in m.g. motoneurones obtained in control cats (filled circles) and cats with local tetanus (open circles). Arrows indicate mean values. Note that Ia e.p.s.p.s elicited by m.g. nerve stimulation in tetanic cats decreased in amplitude while those elicited by stimulation of the l.g.s. nerve, which had been ligated and sectioned peripherally, remained unchanged.

i.p.s.p.s is dependent on the membrane potential. In the present experiments the membrane potentials of motoneurones in which Ia i.p.s.p.s were recorded were not different in control and tetanic animals ($-55·6 \pm 6·7$ mV *versus* $-57·0 \pm 8·9$ mV; $P > 0·5$). Therefore it is unlikely that Ia i.p.s.p.s were not seen in tetanic animals simply because the membrane potential was equal or close to the i.p.s.p. equilibrium potential, unless the equilibrium potential was altered. In some of the motoneurones

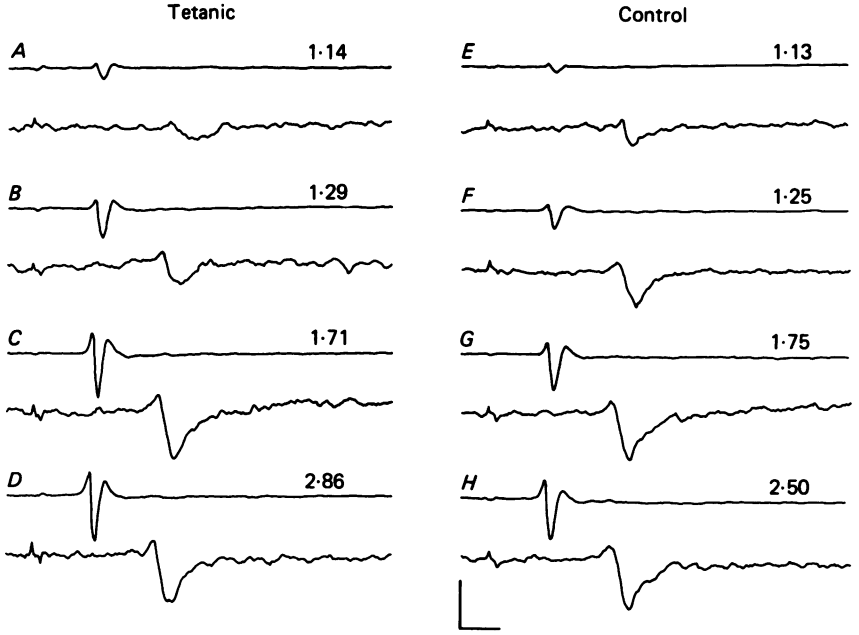

Fig. 5. Examples of the group I incoming (upper traces) and the ascending (lower traces) volleys recorded with ball electrodes placed on the mid line of the dorsal surface of the spinal cord at L_7 and T_{11}, respectively, in a cat with local tetanus 5 days after toxin injection. The m.g. nerves of the left, tetanic leg (left-hand column) and the right, control leg (right-hand column) were stimulated at various intensities, indicated (in multiples of threshold) above each record. The spinal cord was transected at T_{10} and the dorsal column removed over a length of about 5 mm at L_1. Calibrations: 200 μV for the incoming volleys, 10 μV for the ascending volleys, and 1 ms.

TABLE 1. Amplitudes of group I incoming and ascending volleys elicited by stimulation of m.g. nerves of tetanic and control legs

Cat no.	Stimulated nerve*	Max. group I incoming volley (μV)	Ascending volley (μV) (stimulus intensity in multiples of threshold)	
T20	L	191		
	R	196		
T42	L	151	71† (1·7)	
	R	141	80† (1·7)	
T43	L	303	19·1† (1·7)	11·7‡ (1·7)
	R	316	22·3† (1·7)	11·7‡ (1·7)
T44	L	62		6·7‡ (2·6)
	R	66		6·3‡ (2·6)
T45§	L	138		5·6‡ (2·0)
	R	157		4·6‡ (2·0)

All data were obtained 5 days after toxin injection.
* L, left m.g. nerve (tetanic); R, right m.g. nerve (control).
† Recorded from the dissected dorsal quadrant.
‡ Recorded from the dorsal surface of the spinal cord.
§ Ia e.p.s.p. data sampled from this cat are shown by dots in Fig. 2.

of tetanic cats where an i.p.s.p. could not be observed, membrane potential was altered by current injection. This procedure did not reveal the existence of any i.p.s.p.s.

Sural p.s.p.s

Stimulation of sural nerve at a moderate intensity usually produces an initial depolarization and a subsequent hyperpolarization in m.g. motoneurones in control animals (Burke, Jankowska & ten Bruggencate, 1970). The histograms in Fig. 7

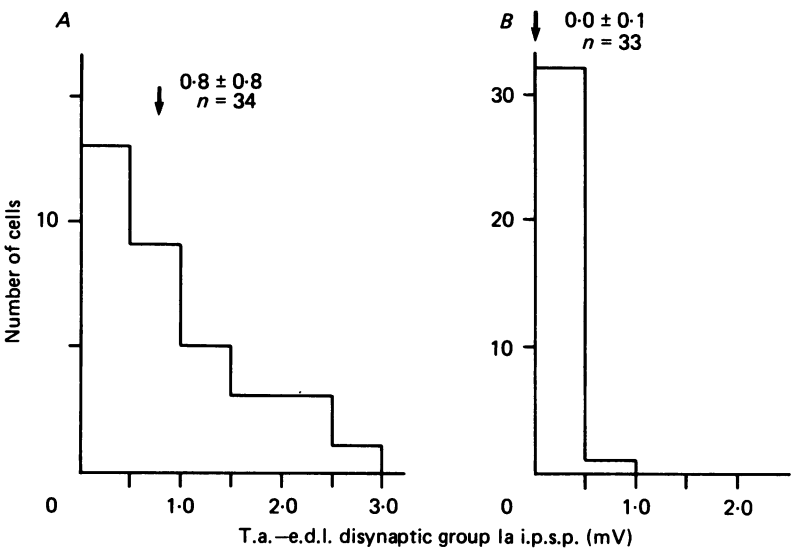

Fig. 6. Histograms showing the distributions of the amplitudes of disynaptic Ia i.p.s.p.s recorded in control cats (*A*) and cats with local tetanus 30–72 h after injection (*B*). Arrows indicate the mean values. Ia i.p.s.p.s are drastically decreased in amplitude in tetanic cats.

illustrate the relative frequencies of three types of sural polysynaptic p.s.p.s recorded in a sample of sixty-two motoneurones with membrane potentials between −55 and −70 mV. If 80% or more of the p.s.p. deflexion was in the depolarizing direction, the p.s.p. was designated an e.p.s.p. (open bar). If 80% or more of the deflexion was in the hyperpolarizing direction the p.s.p. was designated an i.p.s.p. (hatched bar). The others were intermediate or mixed category (designated e.p.s.p.–i.p.s.p.: stippled bar). It is evident that in the control animals most motoneurones studied showed p.s.p.s. either in the mixed or the i.p.s.p. category. In contrast, most of the motoneurones in tetanic animals had p.s.p.s belonging to the e.p.s.p. category (Fig. 1*J*, *O*). Only a few motoneurones showed the mixed, and none of the motoneurones showed the i.p.s.p. type of p.s.p. after toxin injection. The membrane potentials of the motoneurones being compared varied, but there was no significant difference between tetanic and control animals. Therefore, it is unlikely that the differences observed between tetanic and control animals can be accounted for by differences in the level of the membrane potential.

Some motoneurone properties

It has been suggested that tetanus toxin has some effects on the motoneurone itself. Histological examinations of the spinal cord 4–14 days after the onset of rigidity have shown chromatolysis of motoneurones (Tarlov, 1974). It has also been reported that cultured neurones show changes in membrane properties, such as membrane potential, input resistance and threshold current for firing, after exposure to tetanus toxin

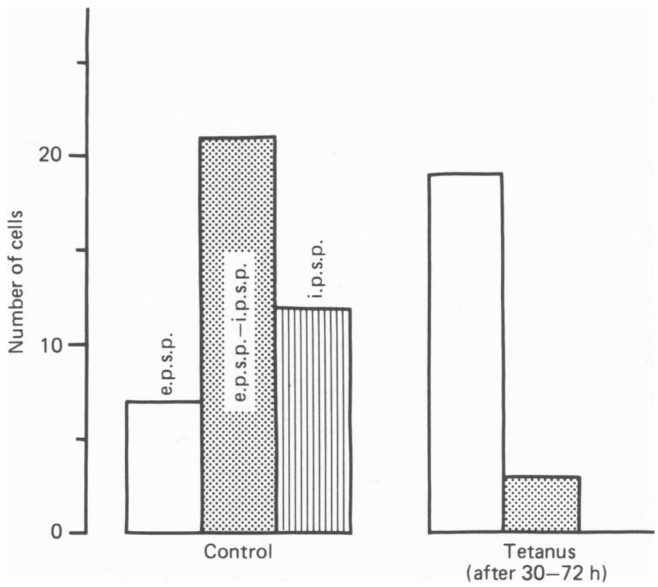

Fig. 7. Histograms of the relative frequency of polysynaptic p.s.p.s elicited in m.g. motoneurones of control cats and cats with local tetanus. Qualitative classification of p.s.p.s according to their predominant deflexion (see text). Note that in local tetanus most of the p.s.p.s belong to the e.p.s.p. category, and none to the i.p.s.p. category.

(Dimpfel, 1979). However, Wiegand & Wellhöner (1979) did not observe any changes in electrical properties of motoneurones in early local tetanus produced by injection of very small doses of tetanus toxin, while the rigidity was evident.

In the present experiments the duration of the after-hyperpolarization, the axonal conduction velocity and the input resistance of motoneurones were measured. The results are shown in Table 2. The duration of the after-hyperpolarization and the input resistance remained unchanged in local tetanus up to 5 days after toxin injection. However, the axonal conduction velocity of motoneurones measured 5 days after toxin injection was slower than that of control animals. The difference was small, but was statistically significant ($P < 0{\cdot}005$). This is in accord with the observation of Mikhailov & Shvarts (1969) that the latency of antidromically stimulated motoneurones increased in the late stage of local tetanus.

Group I incoming volley

Results shown in Figs. 2, 3 and 4 are in accord with previous observations that the monosynaptic reflex and the Ia e.p.s.p.s were eventually depressed following the

injection of tetanus toxin into a muscle (Sverdlov, 1960; Mikhailov & Shvarts, 1969; Terhaar *et al.* 1977). It was suggested by Mikhailov & Shvarts (1969) that the decrease in Ia e.p.s.p. amplitude arises from degenerative processes developing in the motoneurones and the afferent fibres. In the present experiments we compared the size of the maximum group I incoming volley from the tetanic leg with the one from the opposite control leg. The amplitude of group I incoming volleys very much

TABLE 2. Effect of tetanus toxin on motoneurone properties

	Duration of after-hyperpolarization (ms)	Input resistance (mΩ)	Conduction velocity (m/s)
Control	66·3 ± 34·3 (n = 65)	1·0 ± 0·8 (n = 30)	94·1 ± 15·4 (n = 150)
Tetanus (5 days after toxin injection	64·2 ± 18·4 (n = 78)	1·1 ± 0·5 (n = 32)	*89·4 ± 12·7 (n = 203)

Values are mean ± S.D.
* Significantly different (t test, $P < 0·005$).

depended on the recording conditions. Therefore, we chose the contralateral intact side as the control (see Methods). As shown in Table 1 and Fig. 5, we could not see any differences ($P > 0·8$) in group I incoming volleys between tetanic leg and contralateral control leg up to 5 days after toxin injection.

DISCUSSION

The present experiments confirm previous observations that tetanus toxin injected into a muscle eventually depresses the monosynaptic reflex and the Ia e.p.s.p. The lack of differences in the amplitude of group I incoming volleys between tetanus and contralateral control (Fig. 5 and Table 1) and the observation that post-tetanic potentiation could be produced in the depressed Ia synapses of tetanic cats (Kanda, 1981) suggest that the action potentials of Ia afferents do invade the terminal arborization of these afferent fibres. Therefore, the changes observed here cannot be explained by degeneration of Ia afferents. But the latter observation could also mean that some fibres remain unaffected.

Electrophysiological properties of motoneurones, such as the duration of the after-hyperpolarization and the input resistance, were not affected by tetanus toxin (Table 2), although it has been shown that motoneurones contain labelled tetanus toxin (Dimpfel & Habermann, 1973). In three cats lumbosacral motoneurones were examined histologically, and showed no evidence of chromatolysis under our experimental conditions. Furthermore, Ia e.p.s.p.s in response to synergistic, l.g.s. nerve stimulation remained normal when this nerve was sectioned before the injection of toxin into m.g. muscle in order to prevent the l.g.s. nerve from transporting the toxin (Fig. 4). Monosynaptic e.p.s.p.s elicited by stimulation of the ipsilateral ventral quadrant of the thoracic spinal cord were also unaffected (Kryzhanovsky *et al.* 1973; Kanda, 1980*a*). Therefore, it is unlikely that post-synaptic changes, i.e. changes in the general electrical properties of the motoneurone or in the density or sensitivity of post-synaptic receptors, can explain the decreased Ia e.p.s.p. amplitude.

The activity of Ia afferents may be very high because of the high activity of gamma motoneurones (Kano & Takano, 1969). Prolonged hyperactivity may disturb the metabolic turnover of synaptic transmitter storage and release, thus decreasing the amplitude of the Ia e.p.s.p. However, the synaptic transmission between Ia afferents and ascending tract neurones appears to be normal in tetanic cats in the present experiments (Fig. 5 and Table 1). It is thus unlikely that hyperactivity of Ia afferents itself depressed the synaptic transmission between Ia afferents and motoneurones. All these observations suggest that tetanus toxin acts directly on presynaptic terminals of group Ia synapses on motoneurones and blocks the release of transmitter.

In our experiments Ia i.p.s.p.s and the hyperpolarizing component of sural p.s.p. disappeared in the early stage of local tetanus, confirming observations of Sverdlov (1969); Gushchin, Kozhechkin & Sverdlov (1970) and Kryzhanovsky *et al.* (1973). We cannot, however, exclude the possibility that interneurones mediating these reflex arcs are blocked by tetanus toxin, since both of these i.p.s.p.s are produced polysynaptically. Brooks *et al.* (1957) reported that interneuronal activity recorded on the spinal cord surface remained unchanged while various inhibitory reflexes were blocked. This observation led them to the conclusion that tetanus toxin blocks inhibitory synapses on the motoneurone. This has been supported by another experiment in which the Renshaw cell response to antidromic stimulation of motor axons could be seen at a time after toxin injection when the recurrent inhibition was blocked completely (Benecke, Takano, Schmidt & Henatsch, 1977). Therefore, it is likely that disappearance of i.p.s.p.s in the motoneurones observed in the present experiments arises from blockade of inhibitory synapses on the motoneurone.

It has been proposed that the action of tetanus toxin is not specific in terms of the nature of the synaptic transmitter substance, but depends on the accessibility of particular presynaptic terminals (see Kryzhanovsky, 1975; Mellanby & Green, 1981). The present experiments seem to support this notion. The difference in effects on inhibitory and excitatory synapses might be due to a difference in accessibility of those synapses. In this respect, the result shown in Fig. 4 is interesting. Histoautoradiography has revealed that tetanus toxin, after injection into a muscle, reaches the spinal cord by retrograde axonal transport of motor axons (Price, Griffin, Young, Peck & Stocks, 1975; Erdmann, Wiegand & Wellhöner, 1975; Stöckel, Schwab & Thoenen, 1975). It has also been suggested that tetanus toxin migrates trans-synaptically from the motoneurone to inhibitory presynaptic terminals (Schwab & Thoenen, 1976; Price, Griffin & Peck, 1977). It was until now not clear whether the toxin was transported anterogradely by dorsal roots, although dorsal root ganglion cells were clearly labelled (Erdmann *et al.* 1975; Stöckel *et al.* 1975). The results shown in Fig. 4, together with those presented in Fig. 5 and Table 1, suggest that toxin may be transported to Ia presynaptic terminals from the periphery through the dorsal root ganglia but not trans-synaptically from the motoneurone, although trans-synaptic migration of the toxin has been proposed in the case of inhibitory synapses. If so, it can be argued that there is selective uptake of tetanus toxin at some inhibitory synapses on motoneurones and that the action of the toxin on the release of transmitter substances is non-specific once it enters into the presynaptic terminals. If, as has been suggested, the dorsal root ganglion acted as a

barrier, it would be likely that it would take a much longer time for the toxin to reach Ia presynaptic terminals. This would account for the delay and incompleteness of the effect on Ia synapses in comparison with other inhibitory synapses. However, we cannot exclude the possibility that the uptake of tetanus toxin in Ia synaptic terminals depends on transmitter release. L.g.s. Ia synapses were inactive after peripheral severance although some spontanteous release of the transmitter substance must persist in these synapses. This would explain why tetanus toxin does not migrate from m.g. motoneurone to l.g.s. Ia afferent terminals.

The depressed inhibitory and excitatory synapses seem to recover within 3 months of toxin injection (Kanda, 1981). It is not known, however, whether this restoration arises from the formation of new synapses by sprouting collaterals as has been shown in neuromuscular junctions (Duchen, 1973). Finally it should be emphasized that the present results were obtained by injecting tetanus toxin into a leg muscle at a dose of 100 mouse m.l.d. per kilogram of body weight. Different amounts of tetanus toxin or different methods of application may produce results different from those of the present experiments.

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