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TIME COURSE OF FAILURE AND RESUMPTION OF EXCITATORY AND INHIBITORY TRANSMISSION IN THE DENERVATED MUSCLE FIBRES OF THE COCKROACH

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Summary

1. Functional changes in neuromuscular transmission following denervation of the coxal depressor muscle of the cockroach *Periplaneta americana* have been studied by intracellular recording.

2. Miniature postsynaptic potentials, both excitatory (MEPSPs) and inhibitory (MIPSPs), disappeared by about 24 h following nerve section or crush when animals were maintained at 26°C. Generally this was accompanied by the disappearance of excitatory and inhibitory junctional potentials.

3. When animals were kept at 15°C, the onset of failure was delayed markedly, and excitatory transmission ceased a few days before inhibitory transmission, as found previously.

4. Excitatory transmission resumed before inhibitory transmission: in animals maintained at 26°C after nerve crush, MEPSPs reappeared at 11 days and MIPSPs at 28 days. The resumption of transmission was accompanied by the reappearance of junctional potentials.

5. It appears likely that the differences between excitatory and inhibitory transmission, in the timing of failure and of resumption of potentials, are related to the sizes of the axons innervating the coxal muscle of the insect leg.

Introduction

Failure of neuromuscular transmission has been observed a few days after axotomy, with recovery a few days later, in insects (in cockroach by Wood & Usherwood, 1979*a*,*b*; Washio, 1986; in locusts by Clark *et al.* 1979) in a similar fashion to that in the frog (Birks *et al.* 1960).

Insect skeletal muscle fibres are known to be innervated multiterminally and sometimes polyneurally. For example, the coxal depressor muscle (177d and e) of the cockroach is innervated by a slow excitatory motoneurone (Ds) and three common inhibitory motoneurones *via* nerve branch 5r1 (Pearson & Iles, 1971), and possibly by one of the dorsal unpaired median (DUM) neurones (Denburg & Barker, 1982; Tanaka & Washio, 1988). Therefore, it is of interest to see whether

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denervation has different effects on excitatory and inhibitory neuromuscular transmission in these polyneurally innervated muscles. Recently, it has been found in the retractor unguis muscle of the locust *Schistocerca gregaria* that the excitatory innervation disappears a few weeks before the inhibitory innervation (Clark *et al.* 1979). The present report examines the time course of degeneration of excitatory and inhibitory inputs in the cockroach, confirming results of Clark *et al.* (1979) for the locust, and also investigates the time courses for regeneration.

Materials and methods

All experiments were performed on coxal depressor muscles 177d and e of the metathoracic leg of the cockroach *Periplaneta americana* (notations of Carbonell, 1947). While the animal was lightly anaesthetized with carbon dioxide, nerve 5 of the metathoracic ganglion was observed through the transparent thin cuticle covering the nerve between the coxa and the ganglion and was then either cut through or crushed, to axotomize the motoneurones innervating the coxal muscle. Animals were kept at 26°C after the operation unless otherwise stated and the experiments were performed at room temperature (20–23°C).

All electrical signals were recorded intracellularly with micropipettes filled with $2\,mol\,l^{-1}$ potassium citrate. Excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) were evoked by stimulating nerve 5 proximally to branch 5r1 using a pair of fine silver hook electrodes. Activation of inhibitory neurones without activation of excitatory neurones was achieved by stimulation of nerve 5 distal to branch 5r1 (Chesler & Fourtner, 1981). The standard bathing solution for cockroach muscle had the following composition (in $mmol\,l^{-1}$): NaCl, 158; KCl, 10·8; $CaCl_2$, 5; Hepes, 5. The pH of the solution was adjusted to 7·0 with NaOH.

Results

Degeneration

MEPSPs and MIPSPs disappeared almost simultaneously by about 24 h after nerve section or crush, at a post-operation temperature of 26°C (see Fig. 5A,B). Generally, the disappearance was accompanied by the loss of excitatory and inhibitory junctional responses. The time courses of nerve degeneration and regeneration have been shown to depend largely on temperature, and temperature can be used to study the characteristics of each elementary process independently (e.g. Cancalon, 1985). Therefore, the effect of temperatures between 15 and 26°C on time course of failure of excitatory and inhibitory innervation has been investigated.

When animals were maintained at 15°C after nerve section, the disappearance of excitatory transmission began at 6 days and was complete after 11 days (Fig. 1). Inhibitory transmission, however, persisted longer than 11 days. Examples are shown in Figs 2 and 3. Seven days after the nerve section, MEPSPs and MIPSPs

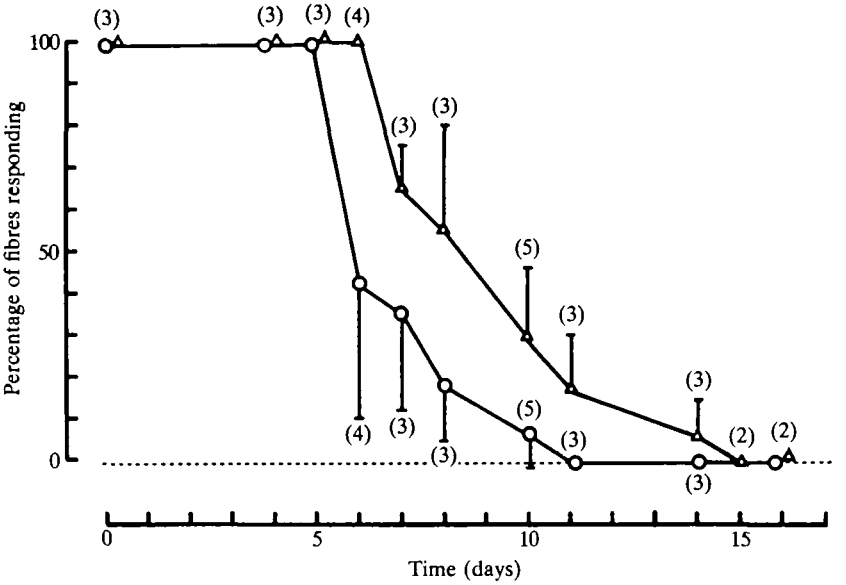

Fig. 1. Time course of changes in excitatory (circles) and inhibitory (triangles) transmission after axons had been cut and animals maintained at 15°C. Abscissa, time in days after the operation. Ordinate, averaged percentage (mean + S.D.) of fibres impaled which showed MEPSPs or MIPSPs from one muscle. At least 20 muscle fibres were tested. Figures in parentheses are the number of muscles tested.

were recorded as depolarizing and hyperpolarizing potential changes, respectively, at a resting membrane potential of 46 mV in coxal depressor muscle 177d (Fig. 2A), as in normal muscle fibres (Washio, 1987). In this figure some examples of EPSPs and IPSPs are also illustrated which are identical with those of normal excitatory and inhibitory junctions. When nerve 5 proximal to branch 5r1 was stimulated with gradually increasing intensity, the EPSP was evoked first, followed by the smaller amplitude, shorter duration depolarizing potential (Fig. 2C) or hyperpolarizing potential (Fig. 2D). When nerve 5 distal to branch 5r1 was stimulated, only IPSPs were evoked (Fig. 2B). At 15°C, MIPSPs and IPSPs remained between 11 and 14 days without MEPSPs and EPSPs (Fig. 3), and ceased completely about 15 days after nerve section.

The times for complete failure of excitatory and inhibitory transmission, for animals at 26°C, 20°C and 15°C, are compared in Fig. 4. With a reduction in temperature, the time for failure of inhibitory transmission was lengthened to a greater extent than the time for failure of excitatory transmission.

Regeneration

Fig. 5 shows the time course of degeneration and regeneration of excitatory and inhibitory transmission in animals held at 26°C after nerve 5 had been crushed (Fig. 5A) or severed (Fig. 5B). As mentioned above, MEPSPs and MIPSPs disappeared almost simultaneously by about 24 h after nerve section or crush at

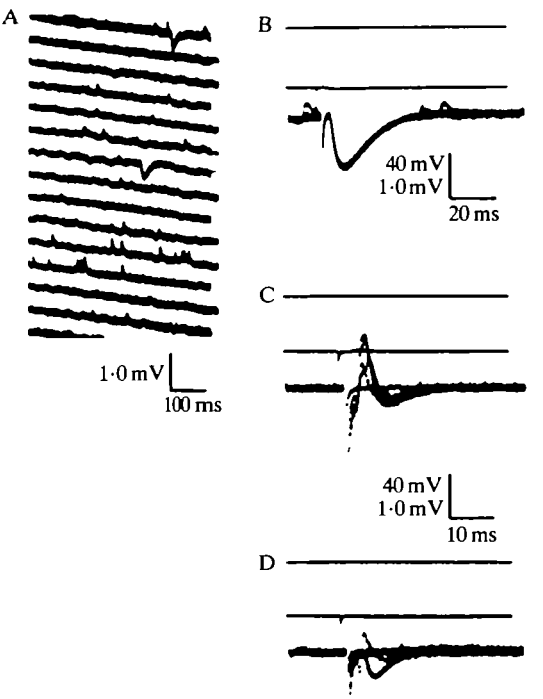

Fig. 2. Synaptic potentials from 7-day denervated coxal muscles (177d). The animal was maintained at 15°C, (A) MEPSPs and MIPSPs; (B) IPSPs and MEPSPs. Nerve 5 distal to branch 5r1 was stimulated. (C,D) EPSPs and IPSPs. Multiple sweeps with gradually increasing stimulus intensities applied to nerve 5 proximal to branch 5r1. Upper trace, reference potential level; middle trace, membrane potential; bottom trace, synaptic potential in B–D. Resting potential: A −46 mV, B −49 mV, C,D −46 mV.

26°C. The earliest reappearance of MEPSPs was observed 11 days following nerve crush, in agreement with previous work (Washio, 1986), but MIPSPs were not observed until 28 days, and then at a very low rate of discharge. Potentials were confirmed as MIPSPs by abolition of the hyperpolarizing potential in Cl^--free saline, or saline containing 10^{-6} mol l^{-1} picrotoxin, and by the persistence of the potential in L-glutamate saline (Washio, 1987). Again, the resumption of MIPSPs was accompanied by junctional potentials.

Examples of the electrical events during regeneration are shown in Figs 6, 7. At 26°C, MEPSPs alone were recorded 22 days after nerve crush, but MIPSPs were not (Fig. 6A). Stimulation of nerve 5 proximal to branch 5r1 did not evoke the IPSP, but evoked the EPSP alone (not shown). In contrast, stimulation distal to branch 5r1 often elicited depolarizing potentials during an early stage of regeneration. These depolarizing junction potentials were not affected by the presence of 10^{-6} mol l^{-1} picrotoxin, and were abolished by the application of L-glutamate, because of the complete desensitization of the glutamatergic synapse (Fig. 6B). Therefore, these depolarizing potentials might be EPSPs (see Fig. 2B,

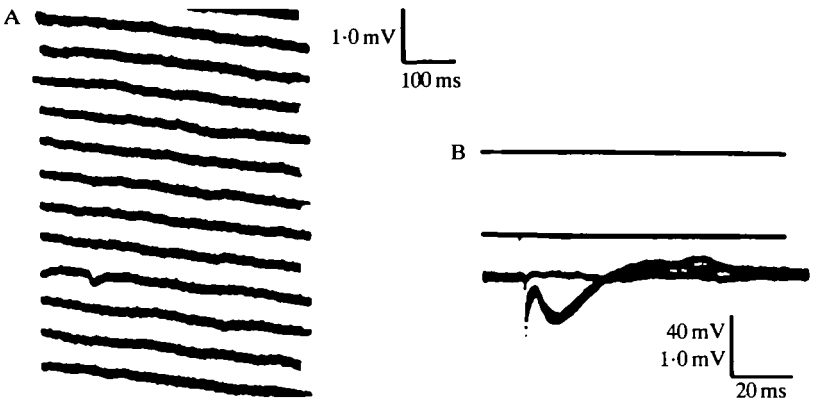

Fig. 3. Synaptic potentials from 11-day denervated coxal muscles (177d) in animals maintained at 15°C. (A) MIPSPs; (B) IPSPs; nerve 5 proximal to branch 5r1 was stimulated. Upper trace, reference potential level; middle trace, membrane potential; bottom trace, synaptic potential. Resting potential: A −48 mV, B −51 mV.

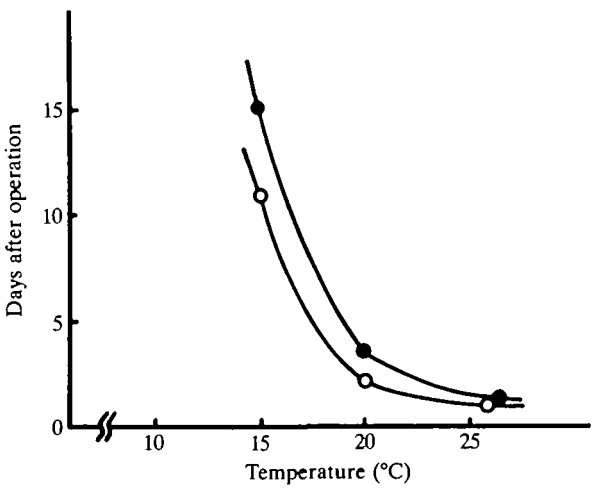

Fig. 4. Effect of temperature on the time course of complete failure (days after operation) of excitatory (○) and inhibitory (●) transmission after nerve section.

also see Discussion). Before the reappearance of MIPSPs, inhibitory junction potentials were occasionally observed upon stimulation of nerve 5 either proximal or distal to branch 5r1, as shown in Fig. 7. At these terminals it seems that the action potentials may invade the terminal membrane and release transmitter. However, it is possible that the frequency of MIPSPs at these terminals is too low to detect the signals.

When the time courses of degeneration and regeneration of transmission at 26°C following nerve crush (Fig. 5A) are compared with those after nerve section

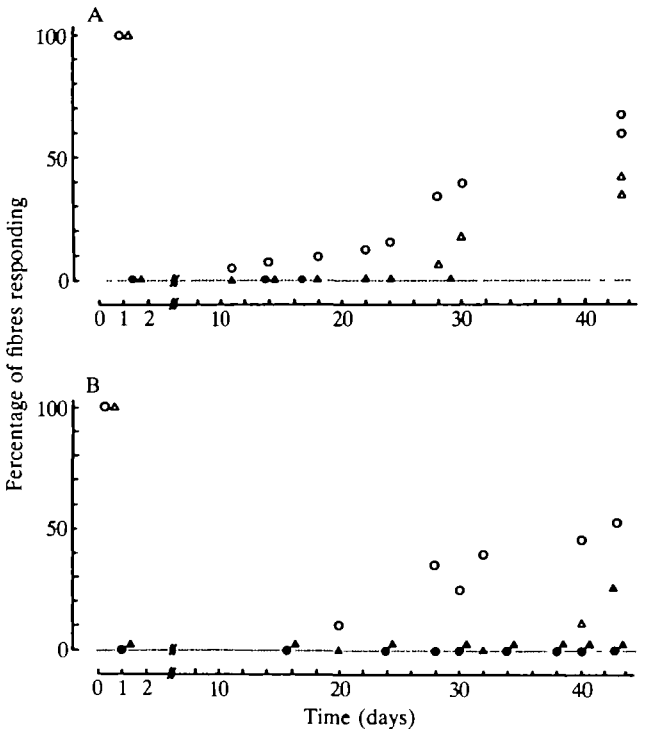

Fig. 5. Time course of changes in excitatory (circles) and inhibitory (triangles) transmission after axons had been crushed (A) or cut (B) and animals maintained at 26°C. Abscissa, time in days after operation. Ordinate, percentage of fibres impaled which showed MEPSPs or MIPSPs from one muscle. At least 20 muscle fibres were tested. Open symbols show fibres with transmission functioning; filled symbols, fibres from which no response to nerve stimulation was observed.

(Fig. 5B), little difference can be seen between the two rates of degeneration. However, reappearance of MEPSPs and MIPSPs after nerve crush occurred more than a week before their recovery following nerve section. This earlier recovery may be because the success of the regenerative process is dependent upon the proximity between the proximal and distal stumps, and the preservation of the connective tissue component of the nerve, together with the glial cells, provides a mechanical guide for growth of regenerating axons (Moore, 1980).

Discussion

After axotomy of the motoneurones innervating the coxal depressor muscle of the cockroach *Periplaneta americana*, the time course of failure and resumption of excitatory transmission could be different from that for inhibitory transmission. There was little difference between times to failure for the two types of transmission when the animals were maintained at 26°C, but as the temperature was lowered, the excitatory output failed before the inhibitory one. The

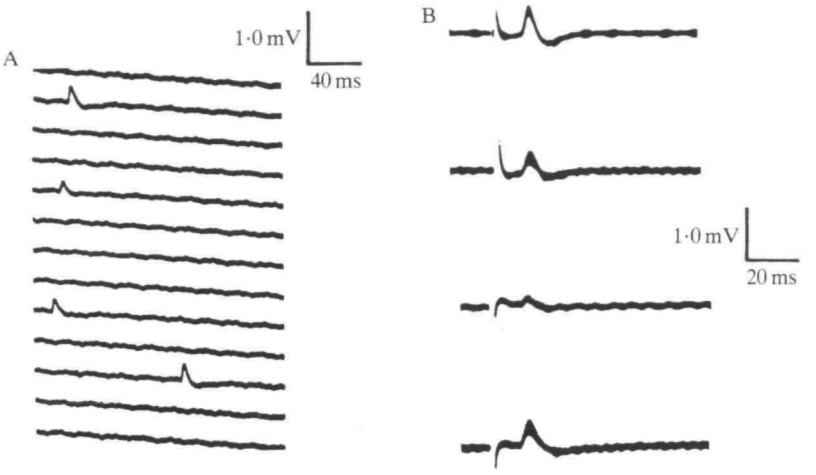

Fig. 6. Regenerating synaptic potentials from the metathoracic coxal muscle (177d) 22 days after axons had been crushed, in animals maintained at 26°C. (A) MEPSPs, but not MIPSPs, were observed. (B) EPSPs recorded by stimulation of nerve 5 distal to branch 5r1. From the top: in normal saline, in a solution containing 10^{-6} mol l^{-1} picrotoxin, 10^{-4} mol l^{-1} L-glutamate, and again normal saline. Resting potential: A −43 mV, B −45 mV.

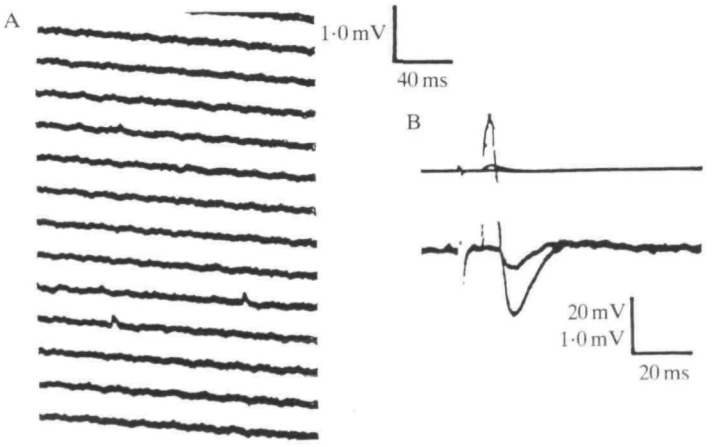

Fig. 7. Regenerating synaptic potentials from muscle 177d 28 days after axons had been crushed in animals maintained at 26°C. (A) MEPSPs, but not MIPSPs, were observed. (B) EPSPs and IPSPs recorded with gradually increasing stimulus intensities applied to nerve 5 distal to branch 5r1. Upper trace is a d.c. recording, lower trace is an a.c. recording. A and B were recorded from the same muscle fibre. Resting potential, −42 mV.

resumption of excitatory transmission took place more than 2 weeks before that of inhibitory transmission at 26°C.

A difference in the time course of failure between excitatory and inhibitory inputs has previously been observed in the retractor unguis muscle of the locust *Schistocerca gregaria*, and it has been suggested that the difference may be due to more rapid degeneration of the larger excitatory axons than the small inhibitory axons (Clark *et al.* 1979). The rate of degeneration of two different sizes of axons innervating the retractor unguis muscle of the locust has also been estimated in a morphological study (Rees & Usherwood, 1972). The larger axon (Ax_1) degenerated about 5 mm every 24 h at 30°C, whereas the smaller one (Ax_2) degenerated about 3 mm in 24 h at this temperature. In the coxal depressor muscle of the cockroach *Periplaneta americana*, a cross-section of the metathoracic nerve branch 5r1, which innervates the coxal depressor muscle, shows that the largest axon is a fast axon, the second largest is slow and the three smaller ones are all inhibitory (Pearson & Iles, 1971). Thus, it seems likely that the larger excitatory axon may degenerate and regenerate more rapidly than the small inhibitory axon in the cockroach coxal muscle.

In contrast, Wallerian degeneration in myelinated fibres of the rat phrenic nerve occurs more rapidly in smaller diameter axons (Lubinska, 1977). In this nerve, Wallerian degeneration of the distal stump has been reported to progress centrifugally by jumping from one internode to another (Singer & Steinberg, 1972; Lubinska, 1982). Thus, it may be that differences between rates of degeneration in insects and mammals are due to the presence of the myelin sheath in mammalian fibres. It is also possible that the ensheathing glial cells may affect the time course of degeneration and regeneration (Singer & Steinberg, 1972).

The present study, and the previous one (Washio, 1986), have shown that the time course of the degeneration and regeneration of neuromuscular transmission of cockroach leg muscles depends on the temperature, the length of the transected distal stump and the size of the nerve innervating the muscle. These results may give some clue to the factor which controls the timing of these events. For instance, the reduction in regeneration of nerves observed at low temperature might be due to decreased axonal transport and a low metabolic rate (Kalia *et al.* 1983). It has also been suggested that a transport system which may translocate the material or signal from the site of injury to the periphery is responsible for determining the duration of the period of end-plate survival after nerve section (Slater, 1966; Miledi & Slater, 1970). Thus, it may well be that the different size of nerves innervating the muscle might alter the rate of axonal transport and, in turn, change the time course of degeneration and regeneration of the transmission.

The cessation of MIPSPs accompanying the failure of inhibitory transmission, and the resumption accompanying recovery, parallels results obtained for the excitatory neuromuscular junctions of the insect (Washio, 1986). The result suggests that the resumed spontaneous release of inhibitory transmitter may be derived from regenerative inhibitory nerve terminals in the same way as for excitatory nerve terminals in cockroach leg muscle (Washio & Nihonmatsu, 1987).

Additional work on the structural changes taking place in the course of regeneration of the inhibitory transmission is required to support this conclusion.

During the process of regeneration of excitatory and inhibitory transmission, somewhat different innervation from normal was often noticed. When nerve 5 distal to branch 5r1 is stimulated, all three common inhibitors are stimulated, but a slow excitatory axon is not. Therefore, no EPSPs are evoked in the fibres of the normal coxal depressor muscle 177d (Fig. 2B, also see Chesler & Fourtner, 1981). However, EPSPs were often evoked by stimulation of nerve 5 distal to branch 5r1 at the early stage of regeneration of excitatory transmission (Figs 6 and 7). The result suggests that there is a possible cross-innervation between excitatory and inhibitory inputs taking place during regeneration.

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