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SPONTANEOUS MINIATURE POTENTIALS IN DENERVATED COXAL MUSCLE FIBRES OF THE AMERICAN COCKROACH

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SUMMARY

1. Functional changes following denervation have been studied by intracellular recording at the neuromuscular junction of the cockroach coxal muscle.

2. Spontaneous subthreshold activity disappeared together with nerve-muscle transmission in about 2 days after nerve section at 26°C. The onset of the failure was mainly dependent on the temperature and also on the length of the transected distal stump. After complete cessation of the miniature excitatory postsynaptic potentials (MEPSPs) for about 3 weeks at 26°C, the miniature potentials resumed at a slower rate. Regeneration occurred faster when axotomy was performed by crushing the nerve rather than by sectioning.

3. Resumption of the MEPSPs was accompanied by response to nerve stimulation. However, hypertonic and hypotonic saline, and excess potassium, failed to increase the frequency of the resumed MEPSPs recorded between 15 and 45 days following denervation.

4. It is suggested that the resumed spontaneous release of transmitter may be derived from regenerating nerve terminals.

INTRODUCTION

Spontaneous miniature endplate potentials (MEPPs) cease a few days after section of the motor nerve to muscle fibres. They are resumed several days later in frog (Katz & Miledi, 1959), but not in cockroach (Wood & Usherwood, 1979*a*) and locust (Usherwood, 1963*a*; Usherwood, Cockrane & Rees, 1968). Reappearance of the discharges is not accompanied by the response to nerve stimulation in frog endplate (Birks, Katy & Miledi, 1960). In the frog, the reappearance of MEPPs is correlated with a close contact of the Schwann cell with the muscle fibre (Birks *et al.* 1960) and the frequency is affected by tonicity (Birks *et al.* 1960; Bevan, Grampp & Miledi, 1976).

The present study describes the time-course of the reappearance of the MEPSPs in cockroach coxal muscle following two types of denervation, and investigates the effect of tonicity changes. A preliminary account of some of these findings has already been published (Washio & Nihonmatus, 1984).

Key words: denervation, regeneration, spontaneous miniature potential, insect muscle.

MATERIALS AND METHODS

All experiments were performed on coxal depressor muscles of the cockroach, *Periplaneta americana*: muscle 178 from the metathoracic leg and 136 from the mesothoracic leg (Carbonell, 1947). The animal was lightly anaesthetized with carbon dioxide during the operation. Nerve 5 on one side of the meta- and mesothoracic ganglia was cut through the transparent thin cuticle covering the nerve between the coxa and the ganglion. The muscle on the other side was tested as the control. In later experiments the motor neurone innervating the coxal muscle was axotomized by crushing nerve 5 with forceps to improve the rate of resumption of spontaneous activity. Animals were maintained at 26 °C after the operation unless otherwise stated, and the experiments were performed at room temperature (20–23 °C).

Excitatory postsynaptic potentials (EPSPs) and MEPSPs were recorded intracellularly with micropipettes filled with 3 mol l^{-1} KCl. The EPSPs were evoked by stimulating nerve 5 with supramaximal square pulses (0·1 ms duration) using a pair of fine silver hook electrodes. The standard bathing solution for cockroach muscles had the following composition (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. Hypertonic and hypotonic salines were prepared by adding 170 mmol l^{-1} sucrose to the standard solution and diluting the standard solution to half strength, respectively.

RESULTS

Denervation by nerve section

After nerve section, spontaneous subthreshold activity ceased in about 2 days at 26 °C and 16 days at 12 °C (Fig. 1). Thus, the time course of the degeneration depended largely on the temperature. During the first 30 h following nerve section at 26 °C, the neuromuscular transmission in the denervated coxal muscles of metathoracic legs remained normal: the 'normal stage' of Ko (1981). The period was found to be shorter in the mesothoracic than in the metathoracic coxal muscle. This result may indicate that the onset of the failure is partly dependent on the length of the transected distal stump (Slater, 1966; Miledi & Slater, 1970). Before the spontaneous discharge disappeared completely, large miniature potentials with high frequency were sometimes recorded in muscles isolated from the animals kept at both 12 °C (Fig. 2) and 26 °C (not shown). The large spontaneous potentials rarely exceeded 2 mV, in agreement with earlier findings (Usherwood *et al.* 1968; Usherwood & Wood, 1973).

After complete cessation of the MEPSPs for about 3 weeks at 26 °C, miniature potentials were again resumed, in eleven out of forty-eight denervated muscles, between 28 and 40 days after nerve section. In these muscles the frequency ranged from 0·05 s^{-1} to 0·65 s^{-1} (0·41 ± 0·17 s^{-1}, mean ± S.D.) in comparison with a mean frequency of 2·85 ± 1·29 s^{-1} in eight normal control muscle fibres. It appeared that most of the miniature potentials in denervated fibres had a slower time course than

those in control fibres (Fig. 3), although a detailed comparison was not made. The increase in time course of the miniature potential might be due to, in part, an increase in muscle input resistance, as suggested by Usherwood *et al.* (1968). The amplitude histogram of MEPSPs from denervated muscle fibres showed a positive skew (Fig. 4) which was the same as that obtained in normal insect muscle fibres (also see Usherwood, 1963*b*; Washio & Inouye, 1978; Washio, 1984).

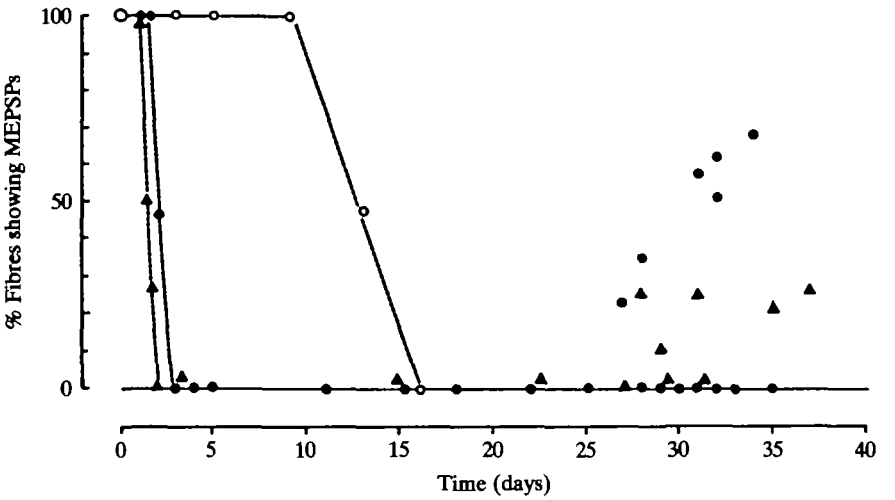

Fig. 1. Time course of changes in spontaneous activity after axons were cut. Abscissa, time in days after the operation. Ordinate, percentage of fibres impaled which showed MEPSPs from one muscle. At least 30 muscle fibres were tested. Data from the metathoracic (circles) and the mesothoracic (triangles) coxal muscle maintained at 26°C (filled symbols) or at 12°C (open symbols).

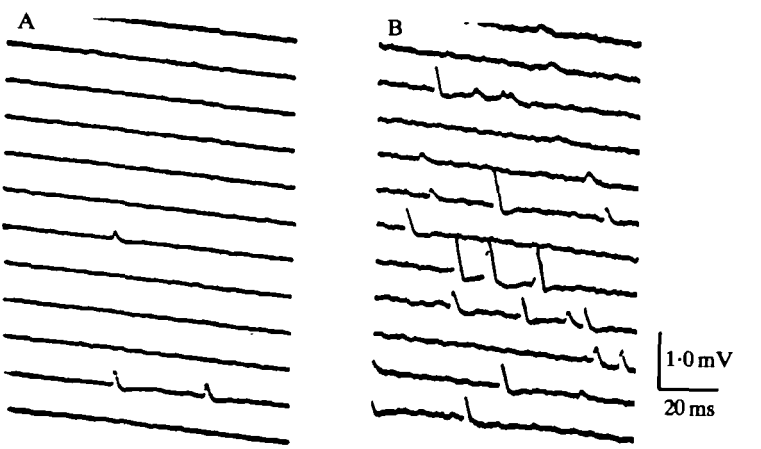

Fig. 2. MEPSPs in normal (A) and 4-day denervated (B) coxal muscles. The animal was maintained at 12°C after the nerve had been cut. The recordings were filtered (high pass, 10 Hz; low pass, 3000 Hz).

Denervation by nerve crush

When the coxal muscle was denervated by nerve crush, degeneration of spontaneous subthreshold activity was the same as that following nerve section, but regeneration occurred sooner (Fig. 5); the earliest appearance of MEPSPs was observed 13 days after nerve crush at 26°C. As far as could be ascertained, the resumption of the miniature potentials was accompanied by a response to nerve

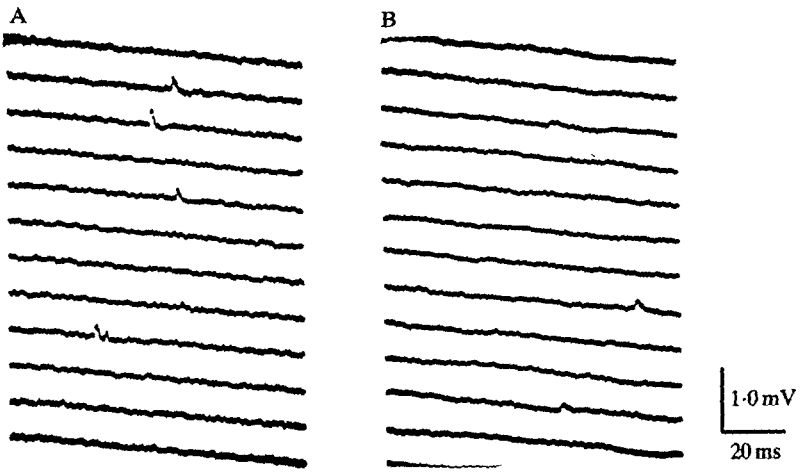

Fig. 3. MEPSPs in normal (A) and 35-day denervated (B) coxal muscles. The animal was maintained at 26°C after the nerve had been cut. The recordings were filtered (high pass, 10 Hz; low pass, 3000 Hz).

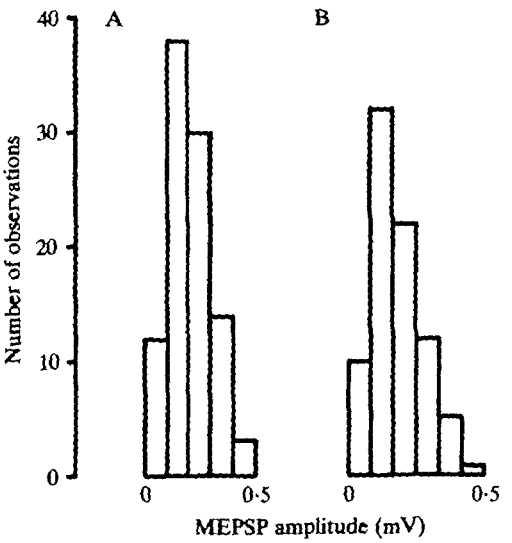

Fig. 4. The distribution of MEPSP amplitude recorded from normal (A) and 41-day denervated (B) coxal muscles. The animal was maintained at 26°C after the nerve had been cut. The mean amplitudes of the MEPSPs were 0·26 ± 0·12 mV (S.D.) in the normal muscle and 0·27 ± 0·14 mV in the denervated muscle. There was no significant difference between them.

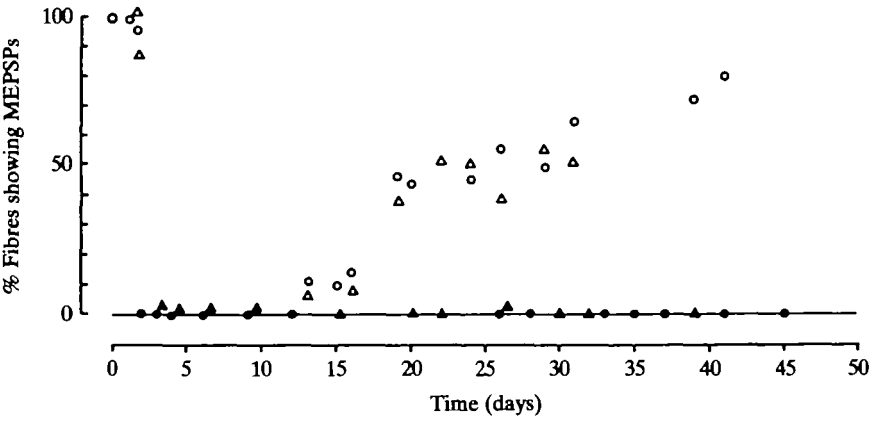

Fig. 5. Time course of changes in neuromuscular transmission after axons were crushed. Abscissa, time in days after operation. Ordinate, percentage of fibres impaled which showed MEPSPs from one muscle. At least 25 muscle fibres were tested. Data from the metathoracic (circles) and mesothoracic (triangles) coxal muscles. Open symbols show fibres with transmission functioning; filled symbols, fibres from which no response to nerve stimulation was obtained.

stimulation (Fig. 5). Also a step-wise fluctuation in the amplitude of the postsynaptic potential (Fig. 6) was often recorded during the regeneration process as well as during the degeneration process (cf. Usherwood, 1963*b*).

Responses of MEPSPs to high K and to tonicity change

The frequency of resumed MEPSPs between 15 and 45 days following denervation was unaffected by high K (Fig. 7), hypertonic solution (Fig. 8), or hypotonic solution (data not shown). In normal preparations, MEPSP frequency was increased by high K (Fig. 7A and Washio & Inouye, 1978), and was increased by hypertonic solution (Fig. 8A and Usherwood, 1961). Hypotonic solution caused a decrease in MEPSP frequency (data not shown). This contrasts with the increase in MEPP frequency that is produced in denervated frog muscles by exposure to hypotonic solution (Birks *et al.* 1960; Miledi & Slater, 1976; Bevan *et al.* 1976).

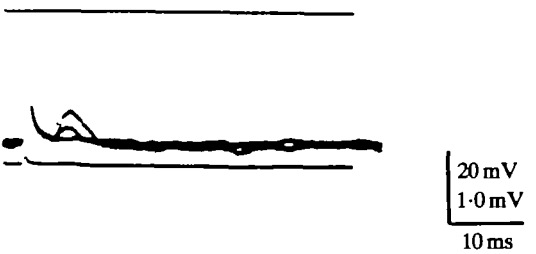

Fig. 6. The postsynaptic responses (middle trace) to nerve stimulation at a frequency of 1 Hz from the metathoracic coxal muscle 77 days after axons had been crushed at 26 °C. The records were obtained by superposition of sweep at 1-s intervals. Upper trace, reference potential level; lower trace, membrane potential.

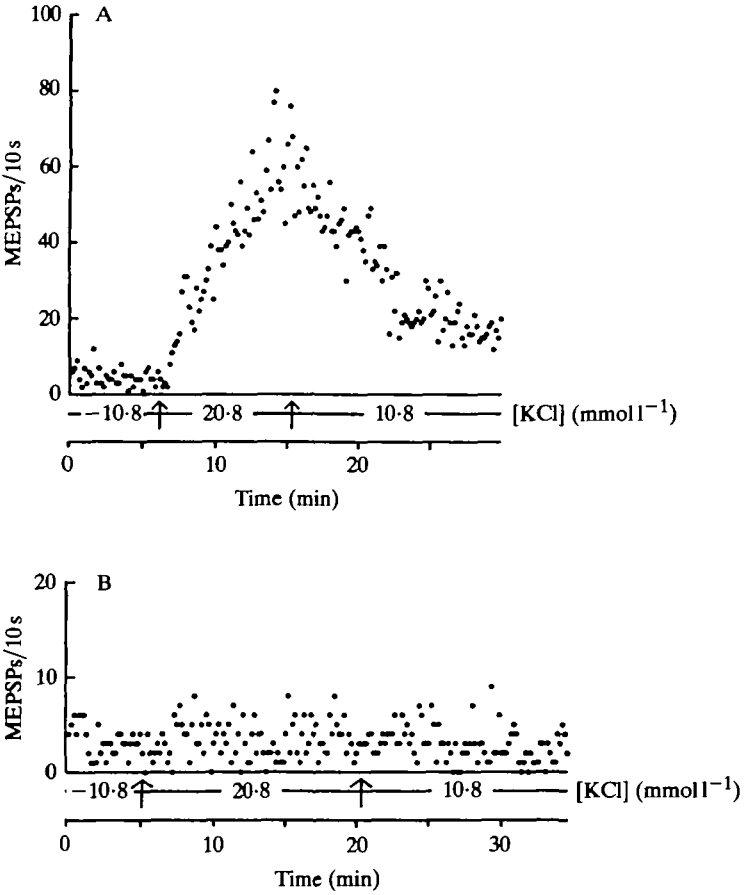

Fig. 7. Time course of the effect of potassium concentration on MEPSP frequency recorded from normal (A) and 27-day denervated (by nerve crush, B) coxal muscles. The extracellular potassium concentration was raised from 10·8 mmol l^{-1} to 20·8 mmol l^{-1} between the arrows.

DISCUSSION

The present study demonstrated the resumption of spontaneous discharges in the denervated coxal muscle of the cockroach, as found previously in cockroach retractor unguis muscle in the process of reinnervation (Wood & Usherwood, 1979*b*). The present results also indicated that the reappearance of the discharges was accompanied by the response to nerve stimulation, in contrast to the finding on the frog endplate (Birks *et al.* 1960). In the frog, the Schwann cell, a type of glial cell, establishes a close contact with the muscle fibre at this stage suggesting that the spontaneous release of acetylcholine from the Schwann cell caused the MEPPs (also see Miledi & Slater, 1968, 1970). Another difference from frog denervated muscles was the effect of tonicity on the release of transmitter. In the frog muscle, hypotonic solutions have been shown to increase the frequency of resumed MEPPs (Birks *et al.* 1960; Bevan *et al.* 1976), whereas such solutions had no effect on the frequency of the resumed MEPSPs in the insect muscle. From these results we may envisage

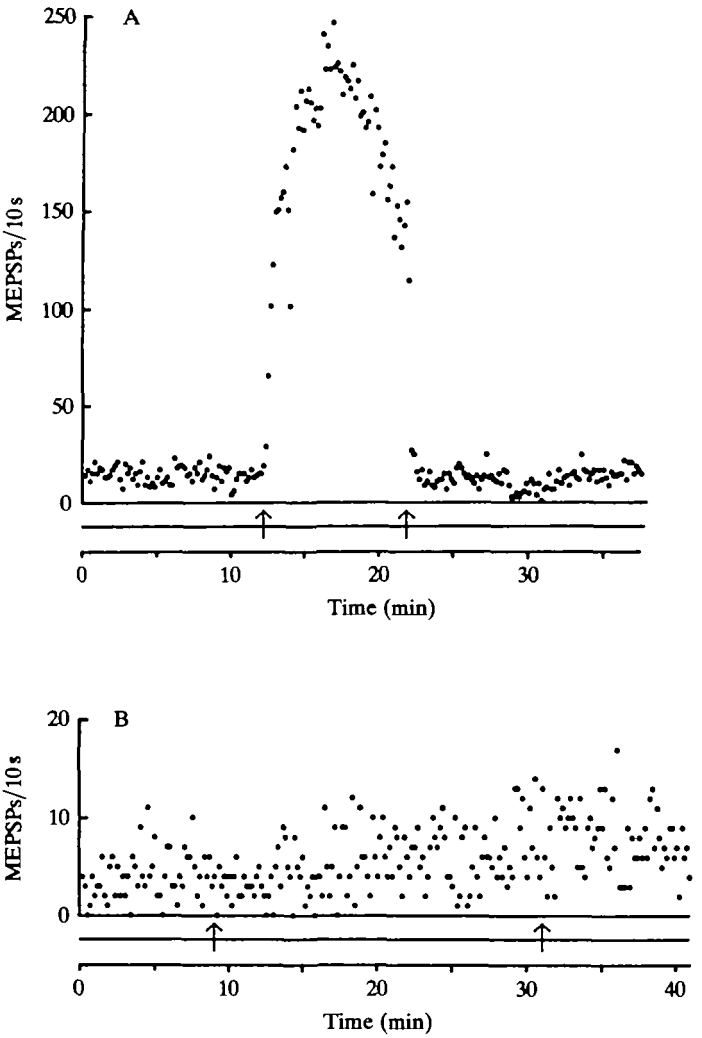

Fig. 8. Time course of the effect of hypertonic solution on MEPSP frequency recorded from normal (A) and 31-day denervated (by nerve section, B) coxal muscles. Arrows indicate the change-over point.

that the spontaneous release of transmitter at the insect denervated neuromuscular junction may be derived from regenerating nerve terminals, not from glial cells with which the cessation of miniature discharges in insect muscle fibres has been correlated (Wood & Usherwood, 1979*a*; Washio & Nihonmatsu, 1984). This notion may be supported by the finding that the amplitude histogram of MEPSPs from denervated muscle fibres shows the same positive skew as that from the normal insect muscle fibre.

On the other hand, raising the external potassium concentration, and application of hypertonic solutions, which strikingly increased the frequency of MEPSPs in normal insect muscles, were ineffective in denervated insect muscle (Figs 7, 8). It has been established that depolarization of the presynaptic membrane opens a gate to

calcium ions and allows influx of the ions (Katz & Miledi, 1967, 1969; Katz, 1969). An increase in the intracellular calcium concentration in the terminals would increase the spontaneous release of transmitter (Katz, 1969; Miledi, 1973; Alnaes & Rahamimoff, 1975). In this context, it appears then that the presynaptic membrane of the denervated insect muscle fibres in which MEPSPs have been resumed does not function well to open a gate to calcium ions in response to agents (raising the potassium concentration) which markedly increase the frequency of MEPSPs in normal muscle fibres. Since extracellular calcium is not required for hyperosmotic neurosecretion (Shimoni, Alnaes & Rahamimoff, 1977), it seems more likely that the release process *per se* might be affected, such that the system cannot respond to change in intraterminal calcium concentrations. It might be worthwhile to point out here that step-wise fluctuations of the EPSP amplitude have been observed during regenerative processes. In general, these fluctuations have been recorded under conditions of low calcium and high magnesium, and thus low quantal content. In this situation the probability of quantal release is very low (del Castillo & Katz, 1954; Christensen & Martin, 1970; Wernig, 1972). Usherwood (1963*b*) also recorded the same type of fluctuations of the postsynaptic potential during the final stage of the breakdown of transmission following denervation of locust leg muscles. Thus, the insect regenerative process might follow an inverse course to the degenerative procedure during the maturation of the junction. Additional work on the structural changes taking place in the course of regeneration is required to establish the origin of the resumed spontaneous subthreshold activity.

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