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Reversal of paralysis in nerve-muscle preparations isolated from animals with hereditary motor endplate disease

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Motor endplate disease (*med*) in the mouse is an hereditary disorder of the skeletal neuromuscular system. Affected animals suffer a 'functional denervation' of skeletal muscle (Duchen & Stefani, 1971). Muscle fibres do not respond to indirect excitation, but motor nerve terminals release transmitter spontaneously. Spontaneous transmitter release can be enhanced by raising $[K^+]_o$ or by exposing muscles to red-back spider venom and functional transmission following indirect stimulation may be restored by 4-aminopyridine.

Introduction Motor endplate disease (*med*) in the mouse is an autosomal recessive disorder characterized by skeletal muscle weakness and wasting. Affected animals are smaller than their phenotypically normal littermates, are weak, and have obvious difficulty in walking. The disorder is rapidly progressive and invariably results in death by 28 days *post-partum* (Duchen & Searle, 1970). Proximal muscles such as the short head of biceps brachii (SH biceps) are severely affected and exhibit muscle fibre atrophy and motor nerve sprouting (Duchen *et al.*, 1967; Duchen & Searle, 1970). Nerve terminals can release transmitter spontaneously but fail to liberate transmitter in response to nerve stimulation indicating that the disorder is a 'functional denervation' (Duchen & Stefani, 1971; Harris & Ward, 1974; Weinstein, 1980). In this communication we describe our attempts to reverse the neuromuscular paralysis.

Methods *Med* mice and their phenotypically normal littermates (aged 15–21 days) were killed by cervical dislocation and the biceps muscles (short head) quickly removed. The isolated muscles were maintained in a bathing solution (composition [mM] K^+ 5.0, Na^+ 150, Ca^{2+} 2.0, Mg^{2+} 1.0, Cl^- 148, $H_2PO_4^-$ 1.0, HCO_3^- 12.0 and D-glucose 11.0), aerated with 5% CO_2 in O_2 and maintained at room temperature.

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Miniature endplate potentials (m.e.p.ps) and indirect action potentials were recorded using standard intracellular techniques.

Results are expressed as arithmetic mean ± standard error of the mean(s.e.mean). Figures in parentheses represent number of observations.

4-Aminopyridine (4-AP) was obtained from Eaton Laboratories and red-back spider venom (RBSV) was a gift from Dr S. Sutherland, Commonwealth Serum Laboratories, Parkville, Melbourne, Australia.

Results The crude homogenate of venom glands of the red-back spider, *Latrodectus mactans*, has been shown to increase m.e.p.p. frequency at the mouse neuromuscular junction (Gorio *et al.*, 1978). M.e.p.p. frequency was recorded before and after exposure to RBSV (0.1 glands per ml, approximately 6 μg protein ml^{-1}). M.e.p.p. frequency increased with release appearing to occur in bursts. As a result of bursting, m.e.p.p. frequency was difficult to measure with accuracy but in both control and *med* muscles, frequency was increased to about 60 s^{-1}. In both phenotypically normal and med muscles the increase in frequency started about 10 min after the venom was applied to the tissue and the effect lasted for a further 20 min or so. There was no early run-down of transmitter release and so it seems unlikely that transmitter stores in *med* motor nerve terminals are unusually small.

Increasing the concentration of K^+ in the bathing fluid from 5 mM to 20 mM resulted in a 40 fold increase in m.e.p.p. frequency in both phenotypically normal (1.2 ± 0.31 [$n = 10$] to 47 ± 6 [$n = 10$] m.e.p.p.s s^{-1}) and *med* (1.1 ± 0.12 [$n = 10$] to 45 ± 4 [$n = 10$] m.e.p.p.s s^{-1}) muscle fibres. Quite clearly, the relationship between K^+-induced depolarization and transmitter release is not impaired in motor endplate disease (see also Duchen & Stefani, 1971).

4-AP is known to increase the amount of transmitter released by nerve impulses, even at endplates blocked by botulinum toxin (Lundh *et al.*, 1977). Four *med*

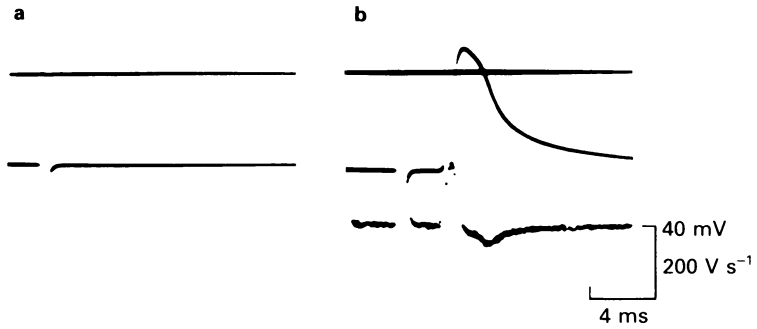

Figure 1 Intracellular records from adjacent muscle fibres in a biceps muscle of a mouse with motor endplate disease. The muscle fibres failed to generate an action potential in response to indirect excitation (a), but became excitable in the presence of 4-aminopyridine (b): top trace = zero potential; middle trace = voltage; bottom trace = 1st derivative of voltage response.

muscles were exposed to 4-AP (0.1 mM). In normal bathing fluids none of the muscles generated any form of twitch response following nerve stimulation. In individual muscle fibres, only 2 out of 40 fibres tested generated any form of evoked synaptic potential. In the presence of 4-AP in all four med muscles a postjunctional response was obtained; 22 out of 86 fibres tested generated either an action potential or an endplate potential, and the muscles twitched in responses to nerve stimulation. Some typical results are illustrated in Figure 1.

Discussion This is the first demonstration that the failure of neuromuscular transmission associated with hereditary motor endplate disease can be reversed.

The precise mode of action of 4-AP is not clear, but it seems that Ca^{2+} influx into the nerve terminal is increased during the depolarization initiated by the invading nerve action potential. The influx of Ca^{2+} is probably increased because 4-AP reduces gK^+, leading to a prolongation of the action potential (Datyner & Gage, 1980; Dunant *et al.*, 1980). If this is the mode of action of 4-AP in *med* nerve-muscle preparations, it would seem that the primary cause of failure in *med* is an inability of Ca^{2+} channels to open during transient depolarization. The increase in m.e.p.p. frequency during exposure to high $[K^+]_o$ suggests that Ca^{2+} channels are opened during prolonged depolarization.

The success of these experiments prompted us to determine whether 4-AP could be used to restore mobility to conscious *med* mice. The drug was administered at doses ranging from 2 to 5 mg kg^{-1} to a total of 4 mice. At no time was mobility improved. It has very recently been shown that *med* mice suffer a defect in the function, and possibly number, of cerebellar Purkinje cells (Pollard, 1984; Dick *et al.*, 1984). In these circumstances, alleviation of the peripheral abnormality may not be totally relevant to the gait disturbance seen in this inherited disease of the mouse.

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