The origin of the repetitive firing of mammalian skeletal muscle after anticholinesterase drugs

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Experiments have been made on the phrenic nerve-diaphragm preparation of the rat maintained *in vitro* in a physiological saline at 37°C (Ferry & Soo Lin Geh, 1977). The bioelectric responses of muscle cells to stimulation of the nerve were recorded with a micropipette filled with 3 M KCl located intracellularly at the endplate region, or with a silver wire electrode or a saline-filled pipette located extracellularly. With extracellular electrodes the response recorded under control conditions at the endplate was a biphasic negative-positive potential due to the initiation of an action potential and its propagation away from the endplate.

After ecothiopate (0.5 μM), the action current was followed by a prolonged inward endplate current which was recorded as a negativity. Repetitive spikes appeared on this negativity 3–5 msec after the initial action current, and with the same biphasic negative-positive potential sequence at first. Later, the repetitive activity, but not the initial action current became mainly positive-going monophasic potentials. At this time an electrode placed 0.2–0.3 mm away from the endplate, along the longitudinal axis of the muscle cell, recorded the initial action current as a triphasic positive-negative-positive potential sequence, with a smaller endplate current and the repetitive activity as a biphasic negative-positive potential.

Records made with an intracellular electrode showed that after ecothiopate, the action potential was followed by an endplate potential prolonged for up to 50 ms. Repetitive action potentials could be grouped into those (a) which arose from, and were associated with, second and subsequent endplate potentials. Repetitive activity of this type was frequently seen in reduced [Mg^{2+}] saline (b) which did not arise from another endplate potential but from the depolarising phase after the peak of the positive after potential of a previous action potential. Repetitive activity in normal physiological saline with [Ca^{2+}] 2 mM, [Mg^{2+}] 1 mM, was mainly of this type.

It is concluded that the repetitive activity in muscle cells *in vitro* bathed in physiological saline most frequently stems from the prolonged endplate potential after ecothiopate. These repetitive action potentials are first generated at the endplate region, but late in the course of ecothiopate action, the locus of initiation of repetitive action potentials moves 0.2–0.3 mm away from the endplate.

This work was supported by the M.R.C.

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Effect of 4-aminopyridine on muscle contractility in the cat

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4-Aminopyridine facilitates neuromuscular transmission by increasing the evoked release of acetylcholine (see, for example, Lundh, 1978). It is used as an anticurare agent in Bulgaria (Stoyanov, Vulchev, Shturbova & Marinova, 1976), and has been shown to restore transmission in the Eaton-Lambert syndrome (Lundh, Nilsson & Rosén, 1977; Agoston, van Weerden, Westra & Broekert, 1978). The compound may also enhance skeletal muscle contractility through a direct action on the muscle fibres, although there appears to be a species difference with regard to this effect. It is reported to be very effective on the rat diaphragm (Bowman, Khan & Savage, 1977), only slightly so on the chick biventer cervicis (Bowman, Harvey & Marshall, 1977), and ineffective on the rabbit tibialis anterior muscle (Lemeignan & Lechat, 1967).

Experiments were performed on the tibialis anterior and soleus muscles of cats under chloralose anaesthesia. Isometric twitches and tetani were evoked by stimulation of the motor nerves, or by direct stimulation of the fully-curarized or chronically denervated muscles. Gross muscle action potentials (belly-tendon leads) were recorded simultaneously with the contractions of the indirectly stimulated muscles as an indication of any changes in the number of contributing muscle fibres or of any repetitive firing.

4-Aminopyridine, in doses of 0.5 mg/kg i.v. and above, produced a slowly-developing increase in the

amplitude of the indirectly or directly evoked maximal twitches of the tibialis anterior muscle. The maximal effect (> 100% increase in twitch tension in 8 cats) was produced by doses of 2 or 3 mg/kg i.v. The effect of a small dose (0.5 mg/kg) was slow to develop, taking from 14 to 23 min from injection to the peak of the effect. Once developed, however, the effect persisted for at least 2 h. The effect on the twitch tension of the tibialis anterior muscle was unaccompanied by any change in the amplitude of the gross muscle action potential; nor was there any evidence of repetitive firing. In some experiments, a prolongation of the second wave of the biphasic action potential was detectable. This effect probably reflected prolongation of the individual muscle fibre action potentials arising from the known effect of 4-aminopyridine to block potassium channels in excitable membranes, including those of muscle fibres (Gillespie & Hutter, 1975). 4-Aminopyridine was without effect on the tension of maximal twitches of the soleus muscle. It did not increase the tension of maximal tetani of either muscle. When maximal twitches of the tibialis anterior muscle were depressed by 70–90% by dantrolene sodium (4 mg/kg), 4-aminopyridine (0.5–2 mg/kg) slowly restored the twitches to, or to slightly above, the control amplitude. It is concluded that 4-aminopyridine can enhance the contractility of cat skeletal muscles, and it is suggested that the effect is evident only when activation of the contractile mechanism is submaximal.

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Tachyphylaxis after repeated dosage of decamethonium in anaesthetized man

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In a previous study we used the tetanic and single twitch responses of the adductor pollicis muscle to demonstrate tachyphylaxis with suxamethonium in anaesthetized man (Sugai, Hughes & Payne, 1975). We have now applied this technique to assess the neuromuscular effects of repeated dosage of decamethonium.

Studies were undertaken in a total of 8 anaesthetized patients who had given their informed consent and were about to undergo urological surgery. The methods used have already been described (Hughes, Ingram & Payne, 1976).

Decamethonium was administered in divided doses to 5 anaesthetized patients and the total dose was repeated after full recovery from neuromuscular blockade. Tachyphylaxis developed after the second or third series of injections; the tetanic response was affected more than single twitch and its pattern was more consistent. In 2 of these patients when the same total dose of decamethonium was administered on 3 occasions, blockade of the tetanic response was diminished after the third treatment (Table 1). In the other 3 patients tachyphylaxis was evident after the second series of injections when the total dose administered was at least twice that given in the first treatment (Table 1). In 4 of these 5 patients who were given neostigmine (2.5 mg i.v.), blockade of the tetanic response was antagonised. Furthermore, in 4 patients who received 2% halothane during recovery from decamethonium, the neuromuscular block was potentiated—an effect we had observed previously with competitive neuromuscular blocking agents (Hughes & Payne, 1977).

In contrast, in a group of 3 patients who were given only one series of injections of decamethonium, ie. before tachyphylaxis had developed, blockade of the tetanic response was potentiated by neostigmine and was not affected by 2% halothane.