Regional curare for the reduction of the safety factor in human motor end-plates studied with single fibre electromyography

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SYNOPSIS A method employing six minutes of regional curarization with 0.5 mg D-tubocurarine and single fibre electromyographic measurements (SFEMG) was used in order to study the effect of an additional small dose of D-tubocurarine. The effect of the additional dose was most pronounced in terms of increased jitter, on motor end-plates with the initially highest jitter. The combination of regional curarization and SFEMG jitter recordings offers a sensitive technique for detecting mild effects of various drugs on neuromuscular transmission not detectable with decrement investigations.

Because of the normally high safety factor for neuromuscular transmission as shown in rats (Waser, 1960), cats (Paton and Waud, 1967), and in man, both *in vitro* (Elmqvist *et al.*, 1964) and *in vivo* (Stålberg *et al.*, 1975), mild pharmacological and metabolic effects cannot be detected, so the safety factor for neuromuscular transmission needs to be reduced in order to reveal minor dysfunctions. Minimal further disturbances can then be detected in particular with single fibre electromyographic (SFEMG) jitter measurements (Ekstedt, 1964; Stålberg and Ekstedt, 1973). The positive relationship between jitter and safety factor was demonstrated by (Stålberg *et al.* 1975).

The aim of the present investigation was to study the effect of a small additional standard dose of D-tubocurarine on slightly curarized neuromuscular junctions employing a regional curare method (Torda and Klonymus, 1966; Foldes *et al.*, 1968; Brown *et al.*, 1975; Horowitz *et al.*, 1975) in order to establish a sensitive method for determining the effects of pharmacological agents on the neuromuscular transmission.

METHODS

SINGLE FIBRE ELECTROMYOGRAPHY Single fibre

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EMG recordings (SFEMG) were made with a side-hole SFEMG electrode with a recording surface of 25 μ m diameter. For detailed description of the method see Ekstedt (1964) and Stålberg (1966).

Action potentials from two single muscle fibres belonging to the same motor unit are recorded as a potential pair. The neuromuscular jitter is due to a variability in the neuromuscular transmission time (Stålberg *et al.*, 1971) and is expressed as mean value of the consecutive differences (MCD) of the intervals between the two components making up the potential pairs. The MCD was calculated off-line with a PDP 11-40 computer and with a time interval counter (Hewlett-Packard 5326B). The recordings were stored on analogue tape (AKAI X330). Also the mean value of the interval between the two components was calculated (MIPI=mean inter-potential interval).

The jitter was studied after a preceding regional intravenous dose of D-tubocurarine, employing a method similar to that used in regional intravenous anaesthesia (Bier, 1908; Thorn-Alquist, 1966) and in regional curarization (Torda and Klonymus, 1966; Foldes *et al.*, 1968; Brown *et al.*, 1975; Horowitz *et al.*, 1975). A blood-pressure cuff around the right upper arm was insufflated to 200-250 mmHg and 0.5 mg D-tubocurarine in 20 ml NaCl was injected slowly into the dorsal vein of the right hand. The cuff was deflated after six minutes.

In the first three minutes after the cuff was released there were prominent impulse blockings and only after this time could potentials suitable for con-

FIG. 1 Jitter value (MCD) of a potential pair after pretreatment by 0.5 mg local D-tubocurarine. Each dot represents MCD of 50 discharges. Arrow: injection of additional 0.5 mg D-tubocurarine intravenously five minutes after release of cuff. Solid curve line: fitted mean value; broken line: extrapolation of progressive MCD decline after release of cuff; vertical line: MCD increase after systemic injection of D-tubocurarine.

tinuous recording be obtained. Recordings with a jitter estimated between 30–100 μ s were selected and maintained for one to three minutes, after which time an additional 0.5 mg or 30 μ g/kg (one experiment) of D-tubocurarine was injected into the left antecubital vein. The recording continued for another 12–15 minutes.

The jitter values expressed as MCD for 50 discharges (Ekstedt *et al.*, 1974) were plotted relative to time. The change in jitter after the additional dose of D-tubocurarine was measured as the difference between the fitted maximal value one to four minutes after the injection and a value obtained by extrapolating the preinjection slope of the curve for this time (Fig. 1).

DECREMENT MEASUREMENTS Surface recordings were made from the right and left abductor digiti quinti muscle respectively at electrical supramaximal stimulation (Medelec NS6) of the ulnar nerve at the wrist at 2 Hz and pulse width 0.1 ms. The recordings were made during a period of 28 to 36 minutes. During the first six minutes the right arm was partially curarized with 0.5 mg D-tubocurarine regionally. In two subjects an additional 0.5 mg D-tubocurarine was given systemically three to five minutes

after cuff release, two others received 30 μ g/kg, and two were not given the additional dose. The six-minute period for the cuff was chosen as a significant decrement (more than 10%) and earliest signs of clinical weakness were found to begin after about four minutes. The decrement was measured as the percentage amplitude reduction of the negative peak between the first and the fourth response. In two additional experiments, the effects of six minutes ischaemia without drug were tested.

SUBJECTS Investigations were made on 11 healthy subjects aged 25–45 years. Decrement measurements were carried out in five of them. Between two investigations on the same subject there was at least a 24 hour interval. The investigations were done in a warm room, the ambient temperature of which was maintained at 20–22°C.

RESULTS

SFEMG All action potential pairs recorded during the first minute after cuff release showed increased jitter (MCD > 50 μ s) and most demonstrated partial neuromuscular blockings. After

FIG. 2 The effect of 0.5 mg intravenous D-tubocurarine on 11 potential pairs from the right EDC muscle after pretreatment with locally injected D-tubocurarine 0.5 mg (solid lines) and on five non-pretreated potential pairs (broken lines). The effect is more prominent for potential pairs in the pretreated muscles.

eight minutes 60% of the potential pairs showed increased jitter but blockings were uncommon. The subjects usually had mild weakness of the finger extensors during the first five minutes

FIG. 3 The jitter increase (ordinate) after 0.5 mg D-tubocurarine on pretreated (●) and nonpretreated (×) motor end-plates. The abscissa represents the initial jitter value. The effect is more prominent for potential pairs with high initial jitter. The correlation coefficient is 0.86.

after cuff release. The 0.5 mg D-tubocurarine injected intravenously into the left arm usually caused transient eye symptoms, but no additional weakness of the finger extensors.

The dynamic changes in one trial after 0.5 mg D-tubocurarine injected intravenously into the left arm are seen in Fig. 1 and the 11 recordings where an additional 0.5 mg was injected are

FIG. 4 Effect on surface decrement in two subjects (A, B) after 30 $\mu\text{g/kg}$ D-tubocurarine ($\uparrow$) after regional curarization in one arm for six minutes. Recordings from abductor digiti quinti muscle in the pretreated (●) and nonpretreated (○) arm. Supramaximal stimulation of the ulnar nerve at 2 Hz, 0.1 ms pulse width. Dashed line: extrapolated normalization of the decrement without the additional dose.

included in Fig. 2. A positive correlation between the initial jitter (20–75 μ s) and the maximal jitter (27–140 μ s), calculated as the mean of 500 discharges, is evident with a greater increase at higher initial jitter values. The correlation coefficient for the linear regression was 0.86 (Fig. 3). In cases where the jitter exceeded about 80 μ s, occasional impulse blockings were observed.

In a number of recordings repetitive discharges of the potential pairs were observed during the time period five to 10 minutes after cuff release.

The MIPI often showed pronounced changes with a maximal decrease or increase of 800 μ s.

In the experiment in which an additional 30 μ g/kg D-tubocurarine was given, prominent impulse blockings were observed for over 10 minutes.

A number of subjects had difficulty in maintaining a regular innervation rate, particularly after the additional dose. There was no significant change in the shape of the action potentials during the recordings.

SURFACE RECORDINGS In two experiments with ischaemia alone there was no decrement one minute after cuff release. The decrements after ischaemia plus regional D-tubocurarine one minute after cuff release were 70, 60, 30, 27, 27, and 20% respectively. The decrements were halved after 10 minutes in the first three investigations. However, in the fourth, the decrement was normalized after three minutes. In the second and third trials an additional 0.5 mg was injected systemically after 10 minutes. The second dose did not change the progressive normalization of the curves. In the fifth and sixth experiments a systemic dose of 30 μ g/kg was given shortly after cuff release. In the partially curarized arm the slope of the decremental curve was flattened, corresponding to an additional 10% decrement from the expected values (Fig. 4).

In the non-treated arm there were no effects on the decrement with the systemic injection of 0.5 mg or 30 μ g/kg D-tubocurarine.

DISCUSSION

Regional injection of D-tubocurarine (Torda and Klonimus, 1966; Foldes *et al.*, 1968; Brown *et al.*, 1975; Horowitz *et al.*, 1975) was used in the present study as the method for partial reduction of the neuromuscular safety factor. Substances injected into the forearm venous system after application of a cuff around the upper arm diffuse through the vascular bed as shown angiographically (Fleming *et al.*, 1966) and with radio-labelled Transferrin (Brown *et al.*, 1975). The relatively even distribution of the injected D-tubocurarine was indicated in the present investigation by widespread histamine response distal to the cuff and increased jitter values in all investigated sites. The increased jitter was due to D-tubocurarine and not to ischaemia, as the jitter normalized within one minute even after 45 minutes of ischaemia (Dahlbäck *et al.*, 1970).

The technique of regional curarization has been used for studying the effect of neuromuscular transmission with repetitive stimulation in normals (Brown *et al.*, 1975) and in patients with myasthenia gravis (Brown and Charlton, 1975; Horowitz *et al.*, 1975). In normal subjects 15 mg D-tubocurarine given systemically produces no decrement but 0.5 mg regional D-tubocurarine produces decremental response within five minutes (Brown *et al.*, 1975), a feature which was also seen in the present investigation.

When regional partial curarization was employed, as low a dose as 30 μ g/kg (2–3 mg) D-tubocurarine injected systemically affected the decrement in the pretreated arm. However, 0.5 mg D-tubocurarine used similarly did not affect the decrement. With SFEMG jitter measurements it is possible to detect minor neuromuscular disturbances even before impulse blockings occur (Ekstedt and Stålberg, 1969; Stålberg and Ekstedt, 1973) and weakness appears. As small a systemic dose of D-tubocurarine as 0.5 mg has a measurable effect on the jitter (Ekstedt and Stålberg, 1969; Stålberg *et al.*, 1975) and this effect was greatly increased when the tested arm was pretreated with regional curarization. The increase of the jitter was greater for higher initial jitter values (Fig. 3); however, impulse blockings were rare. This small amount of blocking was not sufficient to produce

a significant decrement. In the experiment with 30 µg/kg D-tubocurarine there was a larger jitter increase and impulse blockings were prominent, reflected in an increased decrement.

The high initial jitter after local curarization probably reflects the blockade of a portion of the cholinergic receptors (Naess, 1952; Waser, 1960; Thesleff and Quastel, 1965). Any agent that produces further receptor blockade leads to increase in the jitter greater than that seen without previous partial curarization. Disturbances in impulse transmission are seen in a number of pathological conditions—for example, myasthenia gravis (Elmqvist *et al.*, 1964) and re-innervation (Stålberg and Thiele, 1972). Because of the reduced safety factor in these conditions, there may be more dysfunctions when these patients are given therapeutic doses of drugs that affect neuromuscular transmission such as neomycin (Elmqvist and Josefsson, 1962), diphenylhydantoin (Norris *et al.*, 1964) and others which would not affect normal subjects.

The described method for reduction of the safety factor by means of local partial curarization may be of importance for testing the effect on neuromuscular transmission of various agents. The combination of the method of regional curarization with SFEMG jitter measurements may allow the detection of minimal adverse effects on neuromuscular transmission of drugs at therapeutic levels.

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