Effect of Stretch on Neuromuscular Transmission in a Nerve-supplied Single Muscle Fibre

By

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(*With 6 Text-figures*)

When the skeletal muscle fibre is stretched, a slight decrease in membrane resting potential is observed, and, hence, the threshold of the muscle membrane which receives its stimulus from the nerve and transmits it to the end of the muscle fibre becomes more or less low.

The neuromuscular transmission which is a succession of many events the last of which demands the excitation of the muscle membrane can be blocked in many ways such as by reduction of calcium in the Ringer's solution or by addition of magnesium to it (Locke, 1894; Eccles, Katz and Kuffler, 1942; Engbaek, 1948).

Further, it has been established that this blockage is due principally to an interference with the presynatpic release of the transmitter substance, acetylcholine, whereas the excitability of the muscle or nerve fibre itself is almost unaltered. It has also been confirmed that the depolarization at the junction caused by the release of the transmitter substance is modified by a direct and specific action of Mg- and Ca-ions on the quantity of the substance. This was found by analysis of electrical events at the neuromuscular junction (Del Castillo and Stark, 1952; Del Castillo and Engbaek, 1954: Del Castillo and Katz, 1954).

The object of the present work was to gain some information upon the problem whether the stretch of the muscle fibre would counteract and relieve the blockage by lowering the threshold of the postsynaptic muscle membrane and, therefore, by making the diminished depolarization capable of eliciting the muscular action potential.

Methods

Single nerve muscle preparation : The experiments were performed on fresh nerve-supplied muscle fibres isolated from the *biceps* of the frog *Rana japonica* attached with the sciatic nerve. Mechanical activities and other properties of this preparation have been studied by Tamasige (1957). The morphological aspects of the ending are almost identical with the description presented by Gray (1957). An example of the preparation is shown

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in Fig. 1.

Recording apparatus : Glass capillary microelectrodes filled with 3 mol KCl, external tip diameter less than about 0.5 μ (resistance : 20–30 MΩ), were employed to record the electrical changes at the neuromuscular junction. The microelectrode was mounted on a micromanipulator together with an input stage amplifier (cathode follower with the time

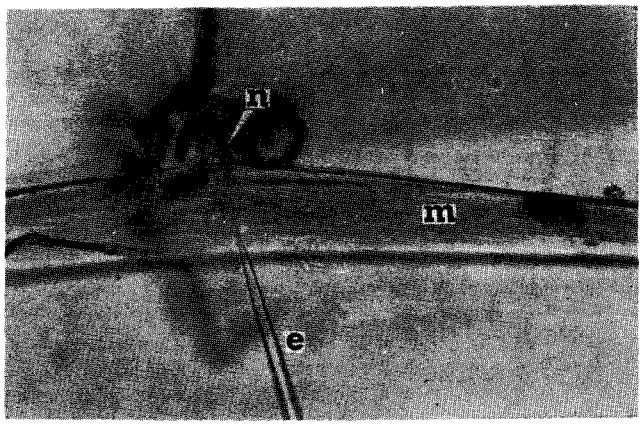

Fig. 1. Photomicrograph of the nerve-supplied single muscle fibre impaled with the microelectrode at the neuromuscular junction. n : nerve fibre, m : muscle fibre. e : microelectrode.

constant of rise 150 μ sec). The input stage of high impedance similar to that described by Nastuk and Hodgkin (1950) and a three-stage balanced d. c. amplifier were used, the final recording being from a cathode-ray tube. Records were made of resting potential, end-plate potential during the blockage and muscle action potential coinciding with the end-plate

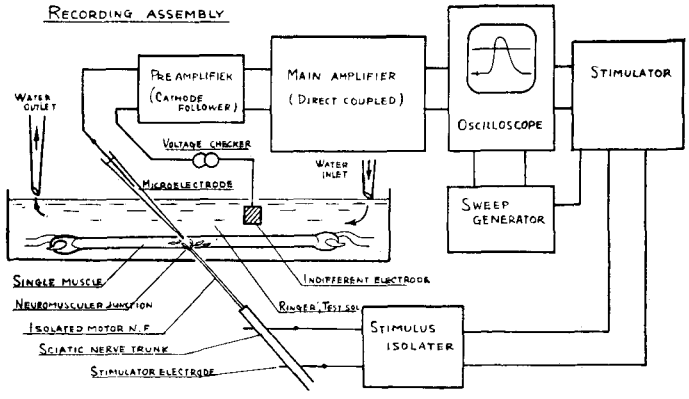

Fig. 2. Schematic arrangement of recording assembly. One of the hooks holding the single muscle fibre at its end is made movable to enable stretching of the muscle fibre.

activity. The indifferent electrode was of a silver-silver chloride type, having a considerable surface area, dipped in Ringer bath.

Stimulation: A square pulse generator triggered synchronously through a delay circuit together with the horizontal sweep circuit of the cathode-ray tube was used to stimulate the nerve fibre supplying the isolated muscle fibre. The pulse (usually 0.2 msec. induration) was fed to a pair of stimulating electrodes of sivler-silver chloride type through an isolating circuit (from the ground) employed to minimize the stimulus artifact.

Solutions: The Ringer's fluid had the following ionic composition, expressed in m.mol/l.: Na, 126.4; K, 2.0; Cl, 127.6; H_2PO_4, 2.0; HCO_3, 1.4. Magnesium ions were added as an isotonic solution of chloride salt, and low calcium Ringer was made by mixing the Ca-free Ringer with the normal Ringer, without changing the tonicity. D-Tubocurarine chloride pentahydrate was made in a concentration of 3×10^{-6}=mol/l in Ringer's solution. All solutions were buffered with isotonic $NaHCO_3$ solution to have pH. 7.2.

Exchange of medium: The experimental trough made with Perspex glass plates was provided with a pair of glass tubes; a water inlet through which the experimental medium was allowed to flow in and a water outlet connected to a suction pump to draw out the excess of the medium. The exchange of the content of the trough was complete in less than 2 minutes, so that the effects of the exchanged media were tested about 5 minutes after the beginning of the exchange, allowing time for adaptation of the preparation to the new solution.

Procedure: The sinlge muscle fibre supplied with nerve was mounted in the trough of Ringer's solution. The nerve was held in air by the stimulating electordes. Both tendons attached to the ends of the muscle fibre were perforated and mounted on a pair of hooks and the fibre was usually kept at equilibrium length. The equilibrium length was defined as that length at which the fibre was just taut when suspended horizontally in Ringer.

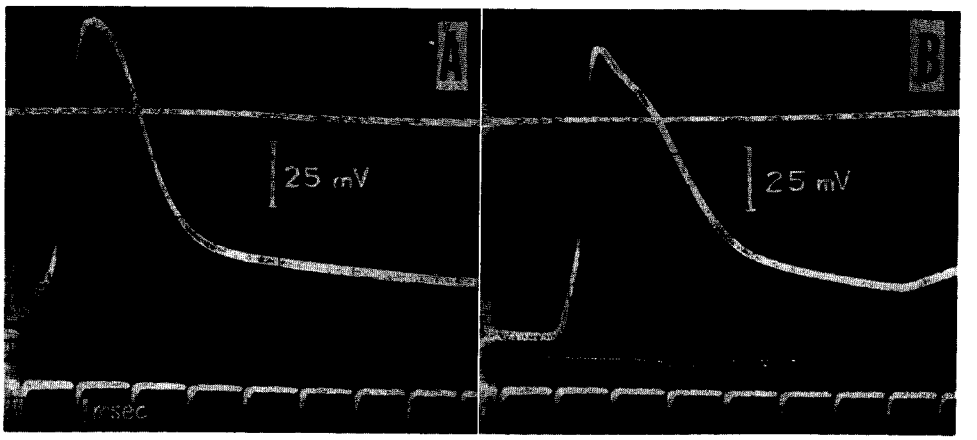

Fig. 3. Typical examples of the muscle action potential and the action potential recorded at the site of the neuromuscular junction. A: muscle action potential recorded at a point 5 mm apart from the junction, B: action potential recorded at the junction. Both records were taken while the muscle was stimulated indirectly through nerve. Note the charcteristic features of the action potential at the junction.

Then the muscle fibre was impaled with the recording electrode at the neuromuscular junction and the resting and action potentials were recorded (Fig. 1).

The Ringer's solution was then replaced with experimental solution with the aid of the exchange devices. The concentration of magneisum or calcium in Ringer's fluid was increased or decreased respectively, until the perfect blockage of transmission was obtained.

When the right concentration was found, the muscle was then stretched in steps of 5 to 10 per cent of the equilibrium length to about 200% of that length allowing sufficient time for adjustment to the new tension at each length. The same procedure of steps was follwed in reverse during release. At each step of the stretch and release the electrical activity of the junction was recorded.

Results

Effect of calcium deficiency The modification of the resting and action potentials and other electrical properties of the muscle fibre by excess or reduced calcium ions have already been extensively studied by Ishiko and Sato (1957); the results obtained in the present investigation were conformatory to their observations. The activity of the neuromuscular junction was greatly modified by the lack of calcium in the Ringer's solution.

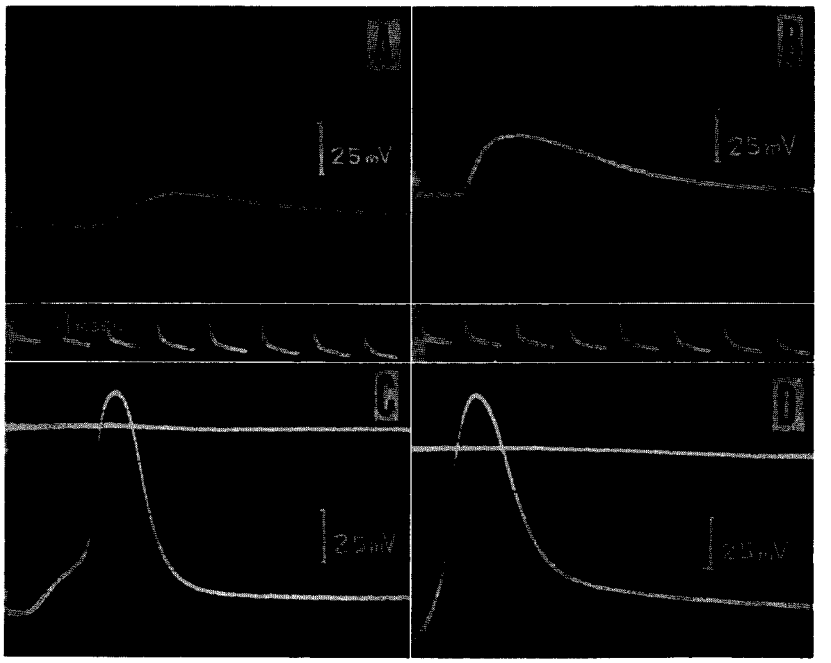

Fig. 4. A series of records of the activity of N-M junction taken at various stages of stretch of a fibre treated with 0.12 mM Ca-Ringer, see also Table 1. A : muscle length 100%, B : 110%, C : 115%, D : 118%.

When the concentration of calcium ions in Ringer was reduced to about one-fifteenth of the normal solution, the muscle fibre never contracted if stimulated indirectly through the nerve attached. The recording of the electrical activity shows that in the time course of Ca-reduction the degree of the end-plate depolarization produced by stimulation of the nerve decreases and becomes insufficient to evoke the muscular activity (Fig. 4, a).

The reduction in the concentration of calcium ions necessary to block critically the transmission differs greatly among individual fibres. The critical concentration ranges one-sixteenth to one-tenth of the normal one, i.e., 0.08–0.13 m. mol/l.

Effect of stretch on the blockage of transmission caused by the Ca-deficiency

Stretching of the muscle fibre in which the transmission is critically blocked produced a marked effect on the depolarization at the postsynaptic membrane: i.e., on the endplate potential. The amplitude of the e.p.p. increased as the muscle fibre was stretched until a propagative muscle spike was set up coinciding with the e.p.p. A first sign of the manifestation in the e.p.p. appeared at the stretch of more than 110% of the equilibrium length.

At a critical degree of stretching which differs from fibre to fibre perhaps because of the difference in the initial amplitude of the reduced e.p.p., the muscle spike fired at the crest of the depolarization (Fig. 4, c). Then as the stretch advanced and the e.p.p. became larger, the muscle spike was elicited at an earlier time. In Table 1 is seen a typical example of the relation between the degree of stretch

Table 1. Effect of stretch on the blockage of N-M transmission with low Ca-Ringer.

Medium	Normal Ringer		0.12 m. mol/l Ca-Ringer			
Fibre length in %. (in cm.)	100 (2.0)	110-118 (2.2-2.35)	100 (2.0)	110 (2.2)	115 (2.3)	118 (2.35)
Resting potential in mV.	90	86	90	86	〃	〃
Action potential in mV.	116	〃	16	28	102	110
Maximum rate of rise of e. p. p. in V/sec.	113	〃	17.5	44.3	40.6	75.0
Critical depolarization in mV.	45	〃	—	—	28	28
Critical depolarization time in msec.	0.3	〃	—	—	1.0	0.5

and the height of depolarization where the muscle spike occurs (critical depolarization) and the time required for the onset of the muscle spike from the start of the e.p.p. (critical depolarization time).

Effect of addition of magnesium on the synaptic transmission

Complete blockage of the transmission was obtained in a Ringer's solution containing magnesium ions more than 10 m.mol/l.

A decrease in excitability has been observed in muscles treated with excess magnesium (Ashkenaz, 1938 ; Maaske and Gibson, 1939). This is due to an effect of magnesium ions on the threshold of the muscle membrane for electrical stimuli. It was found that the threshold depolarization to the production of muscle spike was 53.7±0.8 mV in a solution containing 15 m.mol/l magnesium, whereas in normal Ringer it was only 34.5± 0.67 mV (Del Castillo and Engbaek, 1954).

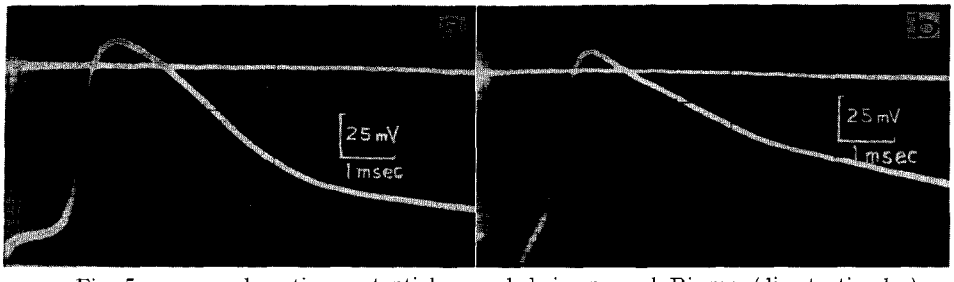

Fig. 5. a : muscle action potential recorded in normal Ringer (direct stimulus). b : muscle action potential recorded in 15 m.mol/l magnesium Ringer (direct stimulus).

Furthermore, in the present experiments a slight deformation of the action potential was observed in the directly stimulated muscle fibre (Fig. 6). The recovery phase of the action potential was prolonged in the presence of magnesium in greater amount than 15 m.mol/l in the Ringer's solution.

Effect of the stretch on the blockage of transmission caused by magnesium

In all twelve experiments where the muscles were stretched up to 200% of their equilibrium length, the stretch failed to relieve the blockage and no remarkable change in the e.p.p. was seen except two cases in which a just perceptible manifestation of the e.p.p. was observed.

A possible explanation of the difference between the blockage by calcium lack and that by magnesium excess will be discussed in a later section.

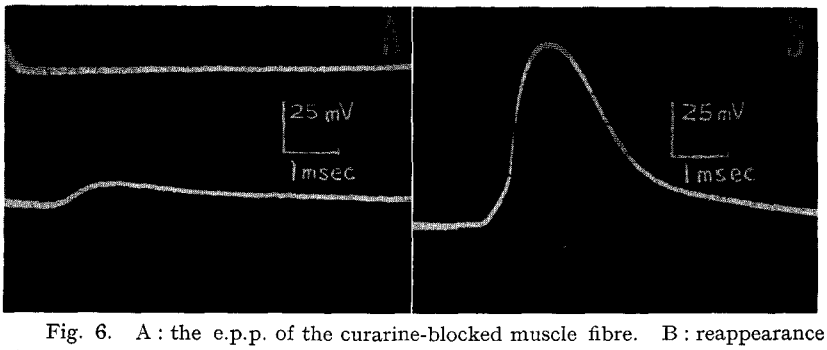

Fig. 6. A : the e.p.p. of the curarine-blocked muscle fibre. B : reappearance of the muscle action potential as a result of 120% stretch.

The blockage produced with curarine

As is well known, curarine has a strong and specific blocking action on the neuromuscular transmission. D-Tubocurarine chloride used in a concentration of 3×10^{-6}mol/l is enough to produce a complete blockage. (The value of critical concentration has a large temperature dependence; the details will be described elsewhere). Therefore, it is of interest to compare the blocking action of this substance with that of the inorganic cations.

The same procedure was employed to examine the effect of the stretch on the preparation blocked with curarine. An example of the record obtained is shown in Fig. 7, where the strotch relieves the blockage as seen in the case of calcium deficiency.

Discussion

There has been a claim to the characteristic features of the action potential recorded at the site of neuromuscular junction (Easton, 1954a, 1954b, 1956). Easton has claimed that these features as seen in Fig. 3a were distortions due to external fields produced by activities of adjacent muscle fibres.

The record of Fig. 3 clearly shows the characteristic features of the action potential recorded at the site of the junction. Since this record was taken in a nerve-supplied *isolated single* muscle fibre as is shown in Fig. 1, Easton's claim should be ruled out.

The mean resting potential of the muscle fibres used here is 87±4.1 mV. The data were discarded in which the muscle shows much smaller value of the potential than the above. This value seems to be quite reasonable in comparison with those reported by many authors (88 mV by Nastuk and Hodgkin, 1950; 90 mV at the junction by Fatt and Katz, 1951; 82±0.6 mV by Jenerick, 1953; 92.2± 0.5 mV by Adrian, 1956; 88.7±2.1 mV by Ishiko and Sato, 1957).

Especially in low calcium Ringer, the muscle tends to become slack quite rapidly when stretched, and, moreover, the muscle membrane seems to become very unstable (Bülbring, Holman and Lüllmann, 1956; Ishiko and Sato, 1957). In many cases the muscle responded by exhibiting two or more successive action potentials at the time of impalement with the electrode. If a train of action potentials appeared the fibre responded no further to the stimulation thereafter, and the resting potential fell down to 30–50 mV. Thus the experiments on the preparations in low calcium Ringer are practically very difficult.

But this is not the case in magnesium treated muscle fibres. As already found (Del Castillo and Engbaek, 1954) the threshold of the muscle fibre increases according to the concentration of magnesium, and in a solution containing 15 m.mol/l magnesium the threshold becomes twice as high as usual.

Perhaps this may account for the impossiblity of recovery from the transmission failure in contrast with the case of calcium deficiency. In the case of calcium, the threshold of muscle fibre decreases as the muscle is stretched and,

in conjunction with the increase of the e.p.p. according to the stretch, the blockage may be relieved whilst the muscular spike reappears.

A possible explanation of the increase of the height of the e.p.p. is as follows: not only the resting potential is decreased by the secondary effect of stretch, but also the threshold current to elicit the muscle spike is decreased to about 60% of the initial strength by the primary effect of stretch on the muscle membrane (Ishiko, 1957, 1958). It has been demonstrated by Ishiko that during the stretch smaller current could produce greater depolarization of the muscle membrane, i.e., I·R drop could be greater than normal. He has attributed this to the increase of membrane permeability to sodium ions. But this explanation seems to be irrelevant to the fact that under stretched condition the resistance of the muscle membrane must be higher if the current required for the depolarization of same magnitude is smaller than normal. Then the depolarization produced by the membrane resistance (R) and the current (I) which flows into the junction would become greater with increase of the stretch, if during the stretch the amount of acetylcholine liberated by nervous activity is not altered and if the amount of electrical charge stays constant, which is carried accompanying with the permeability change induced by the reaction of ACh with its receptor on the muscle membrane (Del Castillo and Katz, 1954, 1956).

The difference between the effect of stretch on the neuromuscular blockage produced by Ca-lack and that by Mg-excess may depend principally on the difference in actions of both ions on the muscle membrane. The results should be considered from this point of view.

There is no evidence of the effect of curarine on the muscle membrane itself at a concentration used here. The effect of stretch may be explained as a direct action on the e.p.p. and the threshold of the muscle membrane, but not on anything else.

It has been reported that the lack of calcium and the excess of magnesium have the similar effect on the release of ACh (Del Castillo and Stark, 1952; Del Castillo and Engbaek, 1954) and they act antagonistically if both ions are added or withdrawn simultaneously.

A problem to be studied further is, what can be expected from the antagonism between magnesium and calcium, when these ions are given simultaneously on the nerve muscle preparation and the muscle fibre is stretched ?

Summary

1. Effect of stretch of a nerve-supplied single muscle fibre on the neuromuscular junction blocked by excess magnesium or reduced calcium Ringer's solution was studied electrophysiologically.

2. Complete blockage of the transmission is observed when the calcium ions in Ringer's fluid were reduced to 0.08 m.mol/l~0.13 m.mol/l. Magnesium

concentration of 10 to 15 m.mol/l is found to be the critical concentration necessary to block the junction.

3. When the muscle fibre blocked with 0.12 m.mol/l Ca solution is stretched up to 110% of the equilibrium length, a first indication of a increase appears in the height of the end-plate potential. When the fibre is stretched over 115% of the equilibrium length, the muscle acition potential reappears coinciding with the e.p.p. and the muscle fibre contracts mechanically.

4. On the muscle fibre blocked by excess Mg, no such effect of stretch is observed.

5. Stretch is found to be effective to relieve the blockage induced by d-Tubocurarine.

6. A possible explanation of the difference between the effects of calcium and magnesium ions is given.

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