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RESTORATION OF NEUROMUSCULAR TRANSMISSION IN SODIUM-FREE HYDRAZINIUM SOLUTION

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Neuromuscular transmission in frog nerve-sartorius preparations, completely blocked by soaking in a sodium-free sucrose solution, was found to be restored by immersion in a sodium-free hydrazinium solution (Koketsu & Nishi, 1958). The present paper describes results of experiments performed to clarify the mode of neuromuscular transmission in this solution.

METHODS

Preparations. Experiments were done mainly with nerve-sartorius preparations, but in some cases with iliofibularis muscles of frogs (*Rana pipiens*) at room temperature (21–24° C).

Solutions. The Ringer's solution consisted of (mM) NaCl 115·6, KCl 2, $CaCl_2$ 1·8, $NaHCO_3$ 2 (pH 6·8–7·0). The sodium-free sucrose solution used throughout consisted of (mM) sucrose 224, $KHCO_3$ 2, $CaCl_2$ 1·8 (pH 6·8–7·0). Hydrazine NH_2–NH_2 (Eastman) was used to prepare the sodium-free hydrazinium solution. The hydrazine solution (224 mM) was first adjusted to pH 6·5 with 224 mM-HCl, and was then made up to 2 mM-KCl and 1·8 mM-$CaCl_2$. The osmotic pressure of this solution, containing approximately 120 mM hydrazine, was practically identical with that of normal Ringer's solution; the freezing point of these two solutions was measured to be −0·465° C. This solution was mixed in various proportions with the 224 mM sucrose solution containing 2 mM-KCl and 1·8 mM-$CaCl_2$, for the purpose of making test solutions containing different amounts of hydrazine. Each test solution was prepared immediately before use, and was again adjusted exactly to pH 6·5 with HCl. Since hydrazine is a weak base and since only the hydrazinium ion is a substitute for sodium ion, the pH of the sodium-free hydrazinium solution should not be higher than 6·5–7·0 (cf. Lorente de Nó, Vidal & Larramendi, 1957). Flame photometry proved that the sodium ion concentration in the test solutions was below 0·01 mM.

Recording system. Isometric muscle responses to nerve stimulation or to directly applied acetylcholine (ACh) were recorded with a mechanical transducer (Grass), an amplifier and electroencephalograph (type D, Offner). Large platinum wires served as leads for extracellular electrical recording from whole tissue. Intracellular recordings were done according to the method of Fatt & Katz (1951). Intracellular potential changes were recorded with d.c. amplification except for spontaneous miniature end-plate potentials which were recorded with a.c. amplification. To measure the resting potential of the end-plate membrane and also the sensitivity of the end-plate membrane to directly applied ACh, the distribution of the surface potential of sartorius muscle was recorded by the experimental arrangement originally described by Fatt (1950) and later modified by Koketsu & Gerard (1956).

Procedure. Preparations were first tested for normal activity in Ringer's solution, and were then soaked in sodium-free sucrose solution (30 min unless otherwise specified) before immersion in a sodium-free hydrazinium solution.

RESULTS

Mechanical responses to nerve stimulation

Neuromuscular transmission in nerve-sartorius preparations was completely blocked by soaking in sodium-free sucrose solution for more than 20–30 min, and no mechanical responses resulted from supramaximal nerve stimulation. When these preparations were then immersed in hydrazinium solution, transmission was restored within 5–10 min, and mechanical responses similar to, or even more powerful than, normal ones were observed. Such restoration of transmission in the hydrazinium solution occurred regardless of the period of previous soaking in sucrose solution, complete restoration being observed even after 180 min soaking. A concentration of 80–90 mM hydrazinium was found to be most effective in restoring and maintaining function. All results herein described were obtained with this concentration. Transmission restored by the hydrazinium solution was not maintained indefinitely; blockage occurred gradually in preparations soaked for an extended time.

An example of the experimental results shown in Fig. 1 was obtained by using 85 mM hydrazine. Transmission, which was completely blocked by soaking in sucrose solution for 20–180 min, showed complete recovery within 10 min in hydrazinium. After 30–40 min transmission block recurred and was complete in about 60–70 min after immersion in the hydrazinium solution. Such late blockage occurs whether nerve stimulations are applied or not. It can in turn be reversed by re-immersion in Ringer's solution.

Electrical responses of muscle fibres

For preliminary observation, electrical responses of the whole sartorius muscle and the nerve twig innervating it were recorded simultaneously with the preparation soaked either in sucrose, or hydrazinium solutions. Figure 2 shows an example of results obtained in such experiments. The electrical activity of the nerve twig was not abolished in the sucrose solution; it only diminished slightly in 60–90 min, and did not disappear even after 180 min in the sucrose solution. This could be because the nerve sheath of the twig was not removed in this, or in any of these experiments. The muscle fibre electrical activity, however, disappeared completely within 20–30 min in sucrose solution. Soon after immersion in the hydrazinium solution, muscle electrical activity reappeared, as is shown in record 3 of Fig. 2. Some characteristic differences, however, were observed between the two electrical activities recorded in the Ringer's and in the hydrazinium solutions. First, the amplitude of the action potential in the hydrazinium solution was much smaller

and less synchronized, compared to that in the Ringer's. Secondly, the muscle response latency was much longer in hydrazinium solution than in the Ringer's, suggesting that either the transmission delay was prolonged or the conduction velocity along the nerve fibre, particularly along its terminal endings, was slowed down.

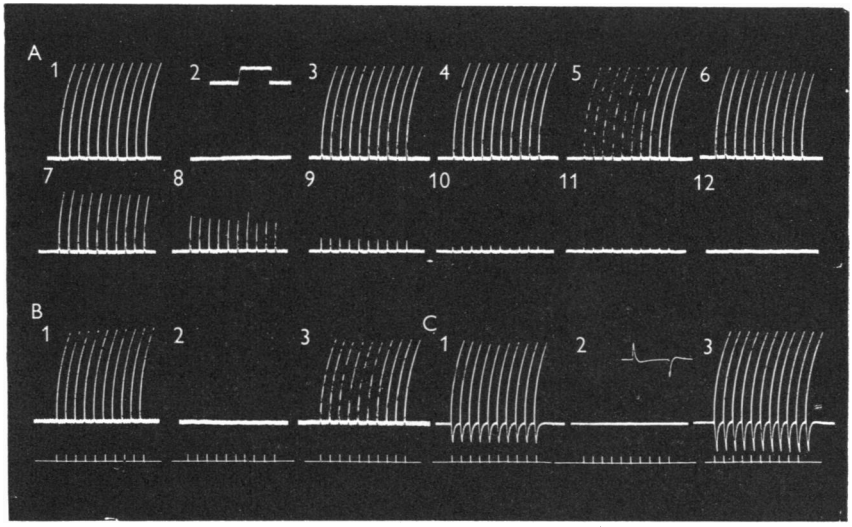

Fig. 1. Isometric mechanical muscle responses produced by supramaximal nerve stimulations obtained from three different nerve-sartorius preparations. 10 nerve stimulations were applied at 1 sec intervals in each record. *A*: Record 1 was obtained in Ringer's solution. Record 2 shows that indirect responses were blocked completely 30 min after soaking in sucrose solution. The preparation was immersed in hydrazinium solution immediately after record 2, and records 3–12 were obtained 10, 20, 30, 35, 40, 45, 50, 55, 60 and 65 min thereafter. B : Record 1 was obtained in Ringer's solution. Record 2 shows that responses were blocked 60 min after soaking in sucrose solution. Record 3 was obtained 15 min after re-immersing the preparation in hydrazinium solution. C: Record 1 was obtained in Ringer's solution. Record 2 was obtained 20 min after soaking this preparation in sucrose solution. Record 3 was obtained 15 min after re-immersion in hydrazinium solution (note that the mechanical responses in record 3 are slightly facilitated compared with those of record 1).

Calibration shown in record 2 (A and C) shows 0·9 g deflexion; and records shown at the bottom are stimulation marks applied at 1 sec intervals; note that record C was obtained by use of a.c. amplifier.

Intracellular potential changes due to nerve stimulation. The resting potential of muscle fibres was slightly lowered in the hydrazinium solution, and there was a tendency for it to fall gradually during prolonged immersion (see Table 1). Muscle action potentials evoked by nerve stimulation were recorded intracellularly within 5–40 min after immersion in hydrazinium solution. Two examples of typical action potentials are shown in records 2 and 3 of Fig. 3, as compared to a control record obtained in Ringer's solution (record 1).

The action potential in the hydrazinium solution resembled that in the Ringer's with the exception that: (1) the spike potentials were practically equal in amplitude to the values of the resting potentials, and did not overshoot and (2) the duration of the spikes was slightly prolonged, with slow rising and falling phases. The muscle spike obtained from the vicinity of the end-plate arose out of the end-plate potential and formed a step on the rising phase (see record 3 of Fig. 3). The spike potential in the hydrazinium solution was followed by a marked negative after-potential similar to that in the Ringer's solution.

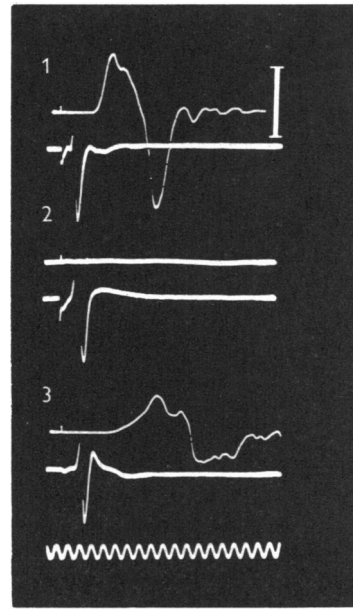

Fig. 2. Each record shows action potentials recorded from the whole sartorius muscle (upper traces) and from the nerve twig innervating it. Single shocks were applied to the nerve. All records were obtained from one preparation; records 1, 2, and 3 were made with the preparation first in Ringer's solution, then 30 min in sucrose solution, and finally 15 min in hydrazinium solution. The muscle action potential disappeared completely in sucrose solution, and recovered in hydrazinium solution, the action potential of the nerve twig was not appreciably affected. Note the prolonged latency and dispersion in the muscle action potential in record 3. Calibration, 10 mV; time marker, 1 msec.

Intracellular potential changes resulting from intracellular stimulation. Two examples of membrane potential changes induced by intracellular stimulation in hydrazinium solution are shown in records 5 and 6 of Fig. 3, as compared to a control record obtained in Ringer's solution (record 4). In comparison to the spike potentials in the Ringer's, those in hydrazinium solution were of smaller amplitude and longer duration, as was also seen in the indirect responses. A significant change in the hydrazinium solution was a threshold

increase (critical depolarization) for the initiation of spike potentials. The mean value of the critical membrane potential for spike initiation, obtained from six different fibres of a preparation immersed in Ringer's solution, was 49 mV, and was 38·6 mV for six different fibres of the same preparation soaked in hydrazinium solution for 10–60 min.

Direct muscle responses could be obtained even after more than 60 min immersion in hydrazinium solution (see record 6 of Fig. 3); those accompanied

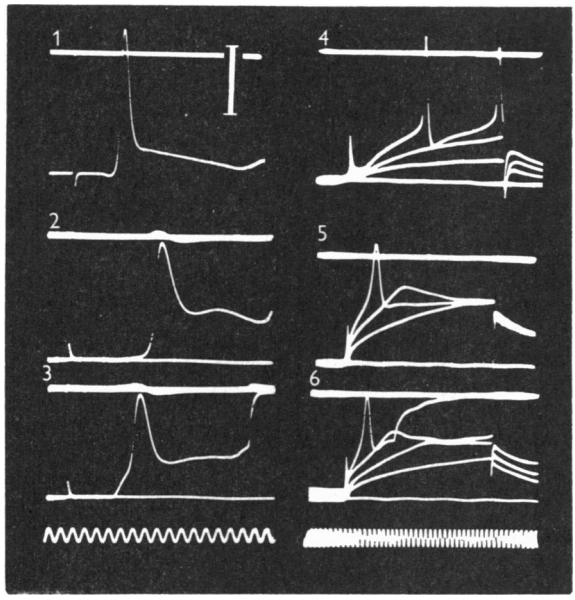

Fig. 3. Intracellularly recorded muscle fibre action potentials evoked by nerve stimulation (records 1–3) and by direct intracellular stimulation (records 4–6). Records 1 and 4 are potential changes in Ringer's solution, the other records are obtained in hydrazinium solution. Records 2, 3 and 5 were obtained between 5 and 30 min after, and record 6 about 80 min after the preparations were immersed in hydrazinium solution. In indirect responses potential changes were evoked by single nerve stimuli, and in direct responses action and electrotonic potential changes were evoked by a suprathreshold and two or three subthreshold stimulations. Local responses seen in records 5 and 6 were produced by just-subthreshold stimulations. The later time course of the action potential was disturbed in several records by muscle contractions. Calibration, 50 mV; time marker, 1 msec.

by a powerful muscle contraction could be easily recorded even after immersion for 180 min. The threshold for the direct responses, however, increased considerably after such extended immersion.

Electrical properties of muscle fibres

The electrical properties of resting muscle fibres in hydrazinium or Ringer's solutions were compared. Two micro-electrodes were inserted close together into a muscle fibre. By using one as a stimulating and the other as a recording

electrode it was possible to calculate the effective resistance and time constant of the membrane from the electrotonic potentials produced by subthreshold, rectangular, direct currents (Koketsu & Nishi, 1957). It is clear that there were no appreciable differences between effective membrane resistance or membrane time constant in the Ringer's and hydrazinium solutions. If the assumption is made that the fibres measured in the Ringer's and those measured in the hydrazinium solution were the same in size and specific internal resistance, then it may be concluded that there was also no appreciable difference

TABLE 1. The resting potential and electrical properties of a sartorius muscle in Ringer's and in hydrazinium solutions. Measurements were taken from six different large fibres in one muscle; first in Ringer's solution, secondly from 10 to 30 min and finally from 90 to 120 min after immersion in hydrazinium

Fibre	Resting potential (mV)	Effective membrane resistance (kΩ)	Membrane time constant (msec)
	Soaked in Ringer's solution		
1	100	135	12
2	100	145	14
3	100	135	15
4	95	110	14
5	100	120	13
6	100	150	12
Mean	99	130	13·3
	Soaked in hydrazinium solution for 10–30 min		
1	88	112	15
2	86·5	114	10
3	96·5	180	10
4	90	114	14
5	87	117	14
6	88	209	15
Mean	89·3	141	13
	Soaked in hydrazinium solution for 90–120 min		
1	87	93	10
2	72	243	12
3	73·5	145	12
4	78	102	8
5	77·5	108	15
6	72	115	12
Mean	81·1	134	11·5

in the specific membrane resistances (Koketsu & Nishi, 1957). This suggests that ionic permeability of the resting muscle membrane was essentially the same in hydrazinium as in Ringer's solution.

End-plate potential

End-plate potentials could be recorded from preparations immersed in a hydrazinium solution containing $3–5 \times 10^{-6}$ g/ml. tubocurarine chloride. A typical example of such potentials is shown in record 2 of Fig. 4, where it is compared with a record obtained in Ringer's solution. End-plate potentials obtained by focal recording (recording from the vicinity of the end-plate)

showed a slower rising phase and somewhat rounded peak as compared with controls in Ringer's solution. Furthermore, the falling phase considered as a passive decay (Fatt & Katz, 1951) apparently shows a longer half-time for decay in hydrazinium solution.

Detection of end-plate potentials became very difficult if immersion in hydrazinium solution was longer than 30 min, although the muscle fibres still responded to nerve stimulation. At this stage the end-plate potentials did not diminish gradually but were abolished abruptly during repetitive nerve stimulation. This suggests that the blockage of transmission was not due to

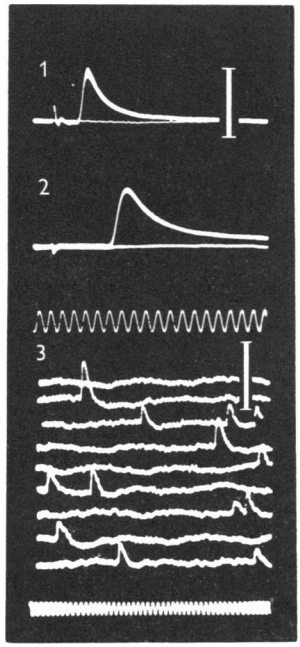

Fig. 4. Typical end-plate potentials recorded intracellularly at the vicinity of the junctional region of a curarized nerve-muscle preparation in Ringer's (record 1) and in hydrazinium solutions (record 2). Note the longer latency and slower rising and falling phases in record 2. Record 3 shows spontaneous miniature end-plate potentials recorded from an uncurarized preparation in hydrazinium solution; successive records were taken by shifting the trace vertically on the face of the oscilloscope. An a.c. amplifier with high amplification was used for this recording. Calibration, 20 mV (records 1 and 2) and 5 mV (record 3); time marker, 1 msec.

a gradual depression of the excitability of the pre- or post-synaptic membrane, but due to blockage of nerve conduction, which probably occurred at the terminal axon. The spontaneous miniature end-plate potential (Fatt & Katz, 1952*a*) was also recorded from uncurarized preparations (see record 3 of Fig. 4). No appreciable differences were observed in the frequency and the amplitude of these potentials obtained in hydrazinium or in Ringer's solutions.

Resting potential of end-plate membrane

The resting potential of the end-plate membrane was measured by recording the distribution of the surface potential of the sartorius muscle (Fatt, 1950; Koketsu & Gerard, 1956). A uniform potential (variation did not exceed 1 mV) could be obtained from two points on the surface of an uninjured sartorius muscle in Ringer's solution. When the preparations were soaked in the sucrose solution for 30–60 min these potential differences were usually produced in an irregular manner along the muscle surface, as described by Fatt (1950). However, when these preparations were immersed in hydrazinium solution

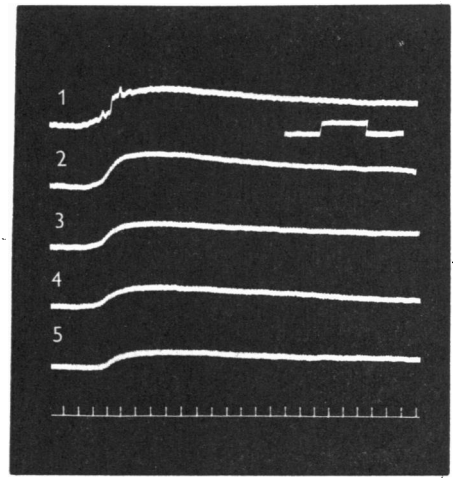

Fig. 5. Isometric mechanical responses of the iliofibularis muscle produced by the application of ACh (5×10^{-5} g/ml.) Record 1 is in Ringer's solution. Records 2–5 were obtained 15, 60, 90 and 180 min after immersion in hydrazinium solution. After taking record 1 the preparation was washed repeatedly in Ringer's solution, and after records 2–5 in hydrazinium solution. Calibration, 0·45 g; time marker, 2 sec.

such unsteady potential differences disappeared and a uniform potential, similar to that observed in the Ringer's solution, was obtained for 10–180 min. This indicates that no appreciable changes were produced in the resting potential of the end-plate membrane, although a slight depolarization probably occurred in parallel with that observed in the resting potential of the whole muscle fibre (see Table 1).

Sensitivity of the end-plate membrane to applied ACh

The tonic mechanical responses of the muscle to directly applied ACh of different concentrations were studied by using the iliofibularis muscle. Visible muscle concentrations were observed on adding ACh of concentrations higher than 1×10^{-8} g/ml. to the Ringer's solution. After soaking in sucrose solution

for more than 30 min, however, no visible contractions were produced, even with ACh at concentrations of 1×10^{-5} g/ml. Upon immersion in hydrazinium solution ACh sensitivity and contractions immediately returned. This recovered sensitivity was well maintained, showing only slight diminution after immersion for 180 min in hydrazinium solution. An example of the contractile response to ACh 5×10^{-5} g/ml., which lasted for 15–180 min in hydrazinium

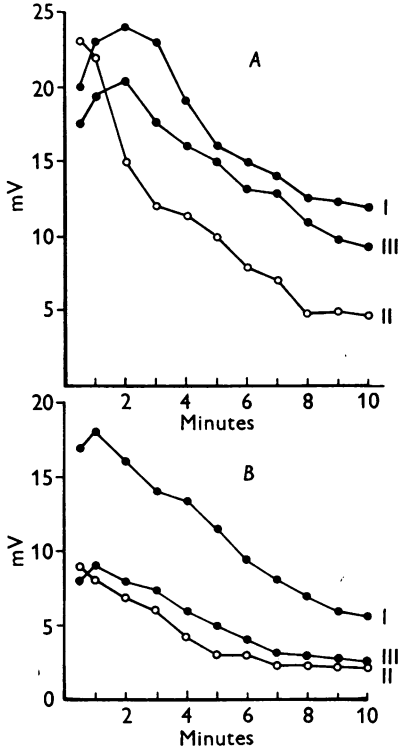

Fig. 6. Sensitivity of the end-plate membrane to directly applied ACh (5×10^{-5} g/ml.). The distribution of the surface potential of the sartorius muscle was measured and the maximum value of the depolarization (ordinate) was plotted against time (abscissa) after application of ACh. Diagrams *A* and *B* were obtained from two different preparations. Measurements were made first in Ringer's solution (curve I), secondly in hydrazinium (curve II) and finally in Ringer's (curve III). Curve II was obtained 15 (*A*) and 90 min (*B*) after immersion in hydrazinium solution. (For details see text.)

solution, is demonstrated in Fig. 5. In this figure, the amplitude of the contraction curve obtained 180 min after immersion in the hydrazinium solution was approximately half of that observed in Ringer's. Such reduction of the contraction, however, usually resulted also in the Ringer's solution, if ACh was applied repeatedly to the preparation.

Sensitivity of the end-plate membrane to directly applied ACh was studied by measuring changes in the distribution of the surface potentials of the

sartorius muscle (Fatt, 1950; Koketsu & Gerard, 1956). The maximum value of the end-plate depolarization was plotted every minute for 10 min after the application of ACh (5×10^{-5} g/ml.) in Ringer's or hydrazinium solution. The conductivity of hydrazinium solution (containing 80–90 mM hydrazine) was measured by use of a Wheatstone's bridge; the ratio of conductance of this solution and of normal Ringer's solution was found to be 0·71:1. Sensitivity was practically identical in these two solutions immediately after application of ACh, but it decreased more rapidly in hydrazinium than in Ringer's solution. Diagram *A* of Fig. 6 shows an example of this experimental series. In this experiment, the distribution of the surface potential was measured after ACh 5×10^{-5} g/ml. was added to the Ringer's solution (curve I). The preparation was then washed in Ringer's solution until the difference in the surface potential disappeared. Next, after soaking for 30 min in sucrose solution, the preparation was immersed in hydrazinium solution, and 15 min later ACh of the same concentration was added and the measurements were repeated (curve II). Immediately after this and repeated washing in Ringer's solution, ACh 5×10^{-5} g/ml. was again added to Ringer's solution and the depolarization was measured (curve III).

Sensitivity, measured after immersion for 30–60 min in hydrazinium solution (partial transmission blockage occurred at this stage), was practically normal in most cases, but showed a slight depression in some preparations. Sensitivity to ACh, however, decreased when the preparation was immersed in hydrazinium solution for 60–180 min. The results plotted in Fig. 6*B* were obtained from experimental procedures used for Fig. 6*A*, except that curve II was obtained 90 min after immersion in hydrazinium solution.

Effects of calcium depletion

It is known that calcium ions are essential for the maintenance of neuromuscular transmission in Ringer's solution (del Castillo & Stark, 1952; Fatt & Katz, 1952*b*). It was found that calcium ions are also essential in sodium-free hydrazinium solution. Neuromuscular transmission restored in hydrazinium solution was readily blocked when immersed in hydrazinium solution from which $CaCl_2$ was omitted. Transmission was not restored when the preparation was immersed in Ca-free hydrazinium solution immediately after soaking in sucrose solution.

The neuromuscular block during calcium deficiency is considered to be due mainly to a reduction in the ACh output from active motor nerve endings (del Castillo & Stark, 1952; Fatt & Katz, 1952*b*). The effect of calcium removal in hydrazinium solution, however, did not seem to be specific for activity of the motor terminal endings. As observed in frog spinal ganglion cell bodies (Koketsu, Cerf & Nishi, 1959*b*), whose excitability was easily eliminated if the calcium ion was absent from the sodium-free media, a number of muscle

fibres lost excitability and no electrical responses were elicited by intracellular direct stimulation in Ca-free hydrazinium solution. The effect of calcium deficiency on the excitability of muscle fibres will be reported in detail in another paper.

DISCUSSION

During the course of the present experiments, it has been confirmed that the excitability of frog muscle fibres cannot be maintained when the external sodium is totally replaced by any one of several quaternary ammonium ions (choline, tetramethylammonium, tetraethylammonium, and tetrabutylammonium) (cf. Hagiwara & Watanabe, 1955). It has, however, been demonstrated that such excitability is maintained if hydrazinium ion is substituted for sodium ion. Thus, under favourable circumstances, frog muscle fibres are capable of maintaining excitability in the absence of external sodium. Crustacean muscle fibres have earlier been shown to maintain their excitability in media where sodium is wholly replaced by different quaternary ammonium ions (Fatt & Katz, 1953; Fatt & Ginsborg, 1958), and mammalian smooth-muscle fibres have been found to maintain their excitability in sodium-free choline solution (Burnstock & Straub, 1958; Daniel & Singh, 1958; W. Woodbury and M. Goto, personal communication). Similar behaviour has been reported for frog nerve (Lorente de Nó, 1949; Larramendi, Lorente de Nó & Vidal, 1956; Lorente de Nó *et al.* 1957; Deck, 1958; Lüttgau, 1958) and for mammalian nerve (Sugaya & Laget, 1958), and for frog spinal ganglion cell bodies (Koketsu, Cerf & Nishi, 1958*a*, *b*, 1959*a*, *b*). The generalization seems to emerge that excitability of various excitable membranes is maintained in media where the sodium ion is replaced by a specific onium ion possessing a favourable affinity for the membrane in question. The possible mechanism of electrical activity in sodium-free media will be fully discussed in another paper.

It is further demonstrated in the present experiment that neuromuscular transmission can be maintained in sodium-free solution, showing that excitability is maintained in the absence of the external sodium not only in the muscle conductile membrane but also in the muscle end-plate membrane. The excitability of the post-synaptic membrane is considered to be different from that of the conductile membrane. The mechanism of production of the end-plate potential has been described as a large, non-selective increase in ion permeability of the membrane, produced by a chemical transmitter (ACh) (Fatt & Katz, 1951). Fatt (1950) reported that ACh still evoked a depolarization of the end-plate membrane after sartorius muscles were immersed in sodium-free glucose or sucrose solution (1–3 hr); the maximum depolarization was about 50% of that occurring in the presence of sodium. According to more recent investigations, however, the depolarizing effect of ACh is reduced or even abolished when sodium is withdrawn from the bath

solution and replaced by sucrose (Nastuk, 1953; del Castillo & Katz, 1955). Furukawa, Takagi & Sugihara (1956) showed that normal depolarization of the end-plate membrane could be produced by externally applied ACh in a sodium-free sucrose solution containing ammonium ion. ACh still reacted with the end-plate receptors and raised the ion permeability of the end-plate membrane, when sodium and chloride had been removed and the cell membrane completely depolarized by substitution of an isotonic potassium sulphate solution (del Castillo & Katz, 1955). These experimental results, as well as the present experiments, suggest that the external sodium ion is not indispensable in order to produce the end-plate responses to a motor nerve volley or to applied ACh. The role of the hydrazinium ion substituted for sodium ion, however, is obscure; it is probable that either the hydrazinium ion is acting in exactly the same way as sodium ion (as a charge carrier) or it increases the permeability of the membrane to calcium ion (or internal anions) during the end-plate potential.

Restoration of sodium-deficient frog nerve axons in hydrazinium solution was initially reported by Lorente de Nó *et al.* (1957). The restoration of neuromuscular transmission in hydrazinium solution shows that motor nerve endings can retain or regain excitability and release ACh in a sodium-free hydrazinium solution.

In view of the present experiments, neuromuscular transmission in the frog apparently can be maintained in the absence of sodium in the external media. It is, however, difficult to disprove the possibility that residual sodium may be retained in the intercellular space, particularly in the extracellular space of nerve fibres, even after prolonged soaking in sodium-free sucrose or hydrazinium solution. There is the further possibility that sodium ions may diffuse out from the interior of the muscle fibres, but it seems difficult to believe that an influx of residual sodium can be responsible for the excitability of the muscle fibre, including the post-synaptic membrane, and even for the excitability of the terminal nerve endings.

Neuromuscular transmission in hydrazinium solution was not maintained indefinitely; blockage occurred finally within 60–70 min. At this stage, however, the individual muscle fibres still maintained excitability and responded to direct intracellular stimulation and also to ACh applied in small concentrations. A slight depression of the sensitivity of the end-plate membrane to applied ACh, observed at this stage, does not seem to be the main reason for the blockage of transmission. It is probably caused mainly by inexcitability of the presynaptic elements. The blockage occurred even when no nerve stimulations were applied, suggesting that it is not caused by shortage of ACh in the motor terminals. That the amplitude of end-plate potentials was not decreased gradually but was abolished abruptly when repetitive nerve stimulations were applied may be accounted for by blockage of nerve conduction. Presumably conduction by the intramuscular nerve fibres was

blocked near the terminal endings. Indeed, the threshold of nerve fibres, as in the muscle fibres, would be increased and conduction blockage could occur at the pre-terminal bifurcation by prolonged immersion in hydrazinium solution.

SUMMARY

1. When neuromuscular transmission in the frog's sartorius has been blocked by 20–180 minutes soaking in sodium-free isotonic sucrose, hydrazinium ions cause transmission to be restored within 5–10 minutes, though ultimately block occurs after about 1 hour in the hydrazinium solution.

2. Intracellular potential changes of muscle fibres induced by nerve and intracellular direct stimulation in hydrazinium solution closely resemble those in Ringer's solution, except that the former shows a slightly longer duration and smaller amplitude. The excitability of muscle fibres was well maintained after complete blockage of transmission occurred.

3. No appreciable changes of the resting potential and electrical properties of the muscle membrane were observed when it was immersed in hydrazinium solution for 180 minutes.

4. The end-plate potential in hydrazinium solution closely resembles that in Ringer's, except for slightly slower rising and falling phases.

5. No appreciable changes in the resting potential of the end-plate membrane were observed during 180 minutes soaking in hydrazinium solution.

6. The sensitivity of the end-plate membrane to applied ACh was well maintained in hydrazinium solution. There was, however, a slight tendency of the sensitivity to fall gradually during prolonged immersion (60–180 minutes).

7. Calcium is essential for the maintenance of neuromuscular transmission in sodium-free solution.

8. Blockage of transmission in hydrazinium solution did not seem to be caused by inexcitability of the post- or presynaptic membranes, but probably by conduction blockage which occurred in the terminal nerve axons.

9. It is suggested that the excitability of different kinds of excitable membranes can be maintained in media whose sodium ions are replaced by onium ions possessing a specific affinity to that membrane.

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