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CHANGES IN POTASSIUM CONCENTRATION AROUND MOTOR NERVE TERMINALS, PRODUCED BY CURRENT FLOW, AND THEIR EFFECTS ON NEUROMUSCULAR TRANSMISSION

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Since the discovery of the spontaneous miniature end-plate potentials (Fatt & Katz, 1952), many conditions which alter their frequency have been found (temperature: Fatt & Katz, 1952; Boyd & Martin, 1956; Liley, 1956*a*; Takeuchi, 1958; osmotic pressure: Fatt & Katz, 1952; Furshpan, 1956; stretch: Hutter & Trautwein, 1956; tetanus: Liley, 1956*b*; Brooks, 1956; Hubbard, 1959; electrotonus: del Castillo & Katz, 1954*b*; Liley, 1956*c*; potassium ion concentration: Liley, 1956*c*; drugs: Brooks, 1956; Kraatz & Trautwein, 1957; Furukawa, Furukawa & Takagi, 1957). These changes in frequency are believed to be due to the alteration of conditions at motor nerve terminals.

At present recording methods do not permit direct measurement of electrical changes at motor nerve terminals. In the experiments to be described the frequency of miniature end-plate potentials was used as an index of changes in the motor nerve terminals produced by several experimental procedures. These were designed to alter the potassium concentration around the nerve terminals and to detect the resultant changes in miniature frequency and neuromuscular transmission. For this purpose inward current was passed through the muscle membrane at the end-plate region. The evidence to be presented indicates that such current reduces the potassium concentration around the nerve terminals and in high-potassium medium produces significant changes in miniature frequency and in transmission. The related problem of the post-tetanic increase in miniature frequency has also been investigated.

METHODS

Experiments were performed on the sartorius muscle of summer frog (*Rana pipiens*) at room temperature (20–23° C). The sartorius muscle was mounted in a lucite (methacrylate resin) chamber with a volume of about 4 ml. Bathing solutions were changed by running at

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least 20 ml. of fresh solution through the chamber. The muscle was left for at least 10 min in each solution before measurement.

Normal Ringer's solution was used of the following composition (mM): NaCl 115; KCl 2·0; $CaCl_2$ 2·0, with added phosphate buffer to keep pH near 7·0. In addition, prostigmine bromide (1×10^{-6} g/ml.) was used to increase the amplitude of miniature end-plate potentials (m.e.p.p.s). In order to increase the concentration of potassium, K_2SO_4 was substituted for NaCl. In some cases the frequency of miniature discharge was increased by raising the osmotic pressure of bathing fluid by adding NaCl or sucrose to normal Ringer's solution (Fatt & Katz, 1952), or by replacing sodium ions by ammonium ions (Furukawa *et al.* 1957).

Micro-electrodes filled with 3 M-KCl were used for potential and current electrodes. The focus of an end-plate was located under microscopical controls by recording m.e.p.p.s of largest amplitude and steepest time course and superficial fibres were used for experiments. The current electrode was connected to a square-pulse generator through a 100 MΩ resistor in series and was inserted within 100 μ of the recording electrode. Electrical changes were recorded by a cathode-ray oscilloscope through a cathode follower pre-amplifier and d.c. amplifier. The membrane potential was measured from the cancelling potential which brought the potential beam back to the original position on the cathode-ray tube. In some cases end-plate current (e.p.c.) was recorded by use of the voltage clamp technique (Takeuchi & Takeuchi, 1959).

RESULTS

Effect of current on the spontaneous m.e.p.p.

When the potassium concentration in the bathing solution was increased, the frequency of spontaneous m.e.p.p.s was increased and usually several minutes elapsed before the change in frequency developed fully. When the preparation was returned from high-potassium solution to one of low potassium, the frequency remained rather high for some time and took about 30 min or more to return to its original value. The frequency of miniature discharge in normal Ringer's solution and the rate of change in frequency when the potassium concentration was altered were different in each end-plate. Relative frequencies in various potassium concentrations as compared to those in normal solution are presented in Fig. 1. Each symbol indicates the mean frequency recorded from an individual end-plate. A marked change in frequency was obtained with increase in potassium concentration to greater than about 5 mM, but smaller changes were observed in lower concentrations. This result is similar to that reported by Liley (1956*c*) with the mammalian end-plate.

After the frequency of miniature discharge became stationary in high-potassium solution, an inward current was passed through muscle membrane by the current electrode, hyperpolarizing the membrane. The current influenced both the amplitude and the frequency of the m.e.p.p. As shown in Fig. 2, when the muscle membrane was hyperpolarized the amplitude of miniature discharge was increased and its frequency decreased. After cessation of the current the amplitude and the frequency returned to their original value. The mean amplitude of the m.e.p.p. had an almost linear relation to the change of membrane potential, as was

observed with the end-plate potential (e.p.p.; Fatt & Katz, 1951). When an inward current was suddenly passed through muscle membrane, the amplitude of the miniature discharge increased rapidly as the membrane was hyperpolarized, but the decrease in frequency developed gradually, becoming nearly constant only about 5–10 sec after the start of current.

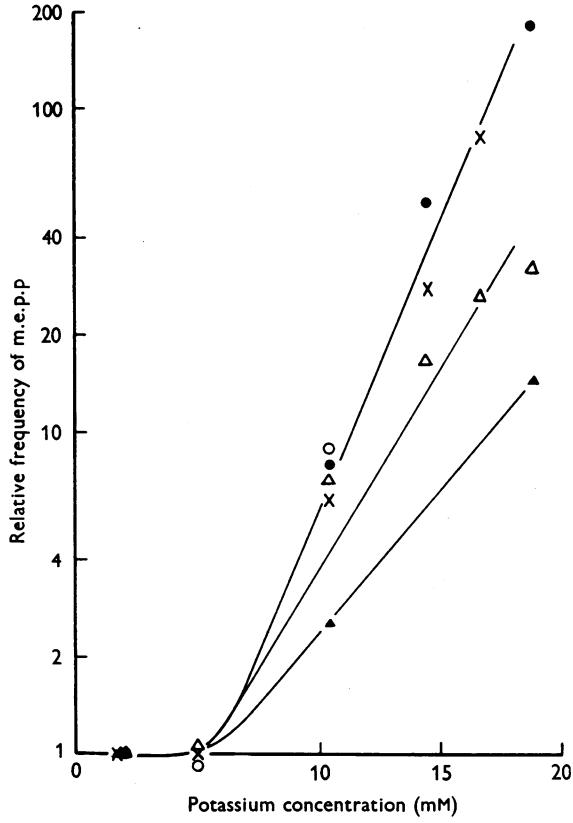

Fig. 1. Relative frequencies in various potassium concentrations as compared to those in normal solution. Each sign indicates mean frequency obtained from an individual end-plate. Semi-log. scale.

After the termination of current the frequency gradually increased and returned to its original value after about 10 sec. In Fig. 3 two examples recorded in 12 mM-K^+ are shown, each point representing mean frequency of miniature discharge for each 400 msec. Beginning at the downward arrows, inward current was passed, which hyperpolarized the membrane by 33 and 38 mV in *A* and *B* respectively. After the start of current about 0·5 sec elapsed before the membrane potential reached a steady hyperpolarized level. During this period the miniature frequency could not be

measured. Following this, however, the frequency change was gradual and reached a fairly steady level after 5–10 sec. At the upward arrows the current was terminated and the frequency gradually increased, returning to near its original value after about 10 sec.

In Fig. 4*A* the mean frequency of the miniature discharge recorded from the same end-plate in various media is plotted against the increase in membrane potential produced by inward current. Filled circles represent the mean frequencies obtained in 10·4 mM-K^+ and crosses and open circles

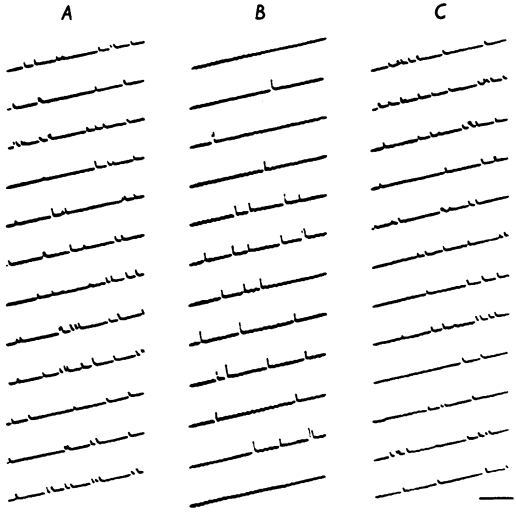

Fig. 2. Effect of current on m.e.p.p. in high-potassium medium (10·4 mM). *A*, before passing current. *B*, while passing current, hyperpolarizing the membrane by 59 mV. *C*, after cessation of current. Time marker, 50 msec.

are those obtained in 14·6 and 16·7 mM-K^+ respectively. The relation between frequency and membrane hyperpolarization was almost linear, with possibly a slight upward concavity.

Several possible explanations of the change in the frequency of the miniature e.p.p. may be considered. (1) A part of the current through the muscle membrane passed through the nerve ending, changing its membrane potential. (2) The current through the muscle membrane altered the sensitivity of the end-plate membrane or interfered with the diffusion of transmitter. (3) The ionic composition of the fluid around the nerve endings was changed by passing current through the muscle membrane. The linear relationship between m.e.p.p. amplitude and membrane potential suggests that the current does not interfere significantly with the sensitivity of the end-plate. The possibility that the electric current disturbs the diffusion of transmitter through the space between nerve terminals and end-plate membrane may also be excluded. Since the

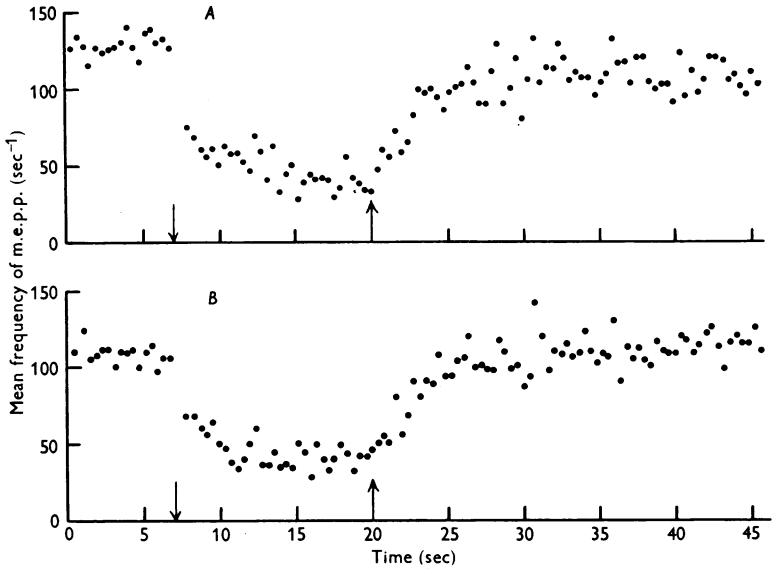

Fig. 3. Time course of the change in miniature discharge frequency by current flow. Each point represents mean frequency of 400 msec in 12 mM-K^+ Ringer's solution. Between arrows the membrane was hyperpolarized by 33 and 38 mV in *A* and *B* respectively. For about 0·5 sec after the start of current frequency was not measured because of instability of the membrane potential.

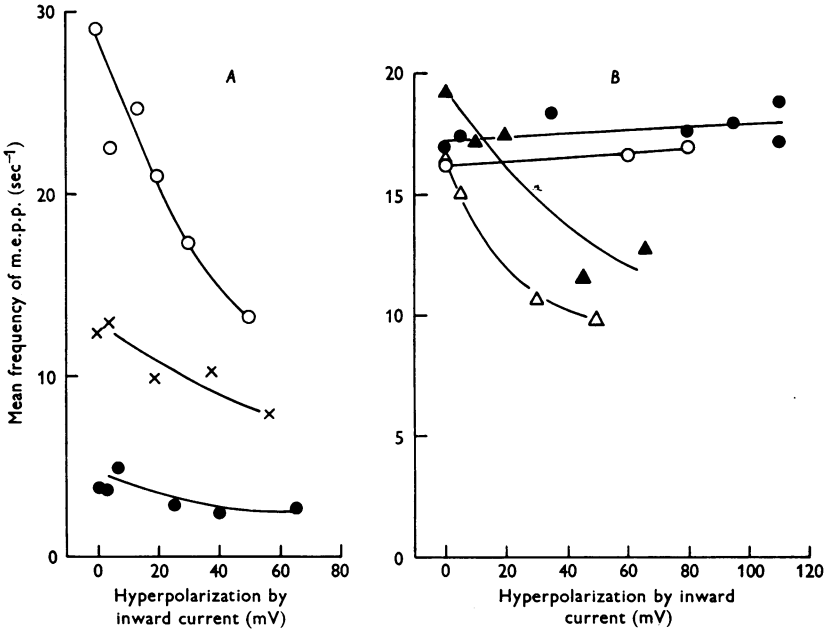

Fig. 4. Effect of hyperpolarization on the frequency of m.e.p.p. *A*: in various potassium concentrations, ○, 16·7 mM; ×, 14·6 mM; ●, 10·4 mM. *B*, ○●, 1·5 times hypertonic solution; △▲, 18·8 mM-K^+.

direction of the current is inward through the muscle membrane and acetylcholine (ACh) is positively charged, the current would not impede the diffusion of transmitter but rather accelerate it.

If the inward current through the muscle membrane were to pass through the nerve ending its direction through the nerve terminal membrane is most likely to be outward. This would be expected to depolarize the nerve terminals and increase the frequency of the m.e.p.p., contradicting the present results. Liley (1956*c*) observed that after the onset of electrotonic polarization of the nerve terminals the frequency attained its final value almost instantaneously. In the present experiments, however, about 10 sec was necessary for the frequency change to develop fully after the start of the current. Furthermore, the frequency change was manifest only in high-potassium medium while when the discharge frequency was increased in hypertonic solution or in ammonium solution, inward current had little or no influence on the frequency of the m.e.p.p.s (cf. Fig. 4*B*). These results exclude the first explanation.

The most probable explanation appears to be that the muscle membrane has a relatively low permeability to sodium ion and that inward current is carried mainly by inward-moving potassium ion and outward-moving chloride ion. The potassium ion concentration in the space between nerve terminals and muscle membrane would be reduced by the flow of inward current, decreasing the discharge frequency of m.e.p.p. If this were the case the amount of change in concentration could be estimated from the frequency change in various potassium concentrations. As shown in Fig. 4*A*, in 16·7 mM-K^+ the current which hyperpolarized the membrane by about 50 mV decreased the frequency from 29 to 13·2/sec. The latter value corresponds to the frequency obtained from same end-plate in 14·6 mM-K^+. Thus in this case it may be supposed that the current decreased the potassium concentration near muscle fibre by about 2 mM. In normal solution inward current had little influence on the discharge frequency. This may be due to the fact that in these circumstances the concentration changes of K^+ that might occur on passing inward current would be expected to have little influence on the discharge frequency (cf. Fig. 1).

The effect of outward current on the discharge frequency was not investigated extensively, because when the membrane was depolarized the amplitude of the miniature discharge became small and measurement of frequency was difficult.

Effect of current on the neuromuscular transmission

The change in discharge frequency of m.e.p.p.s by the passage of inward current appears to result from a change in potassium concentration around

the nerve terminals. In this section the effect of such current on neuromuscular transmission in high-potassium medium was investigated. The amplitude of the e.p.c. clamped at a hyperpolarized potential by a short pulse of about 15 msec was compared with that clamped at same potential by a long pulse (longer than 10 sec). As is shown in Table 1*A*, in a high-potassium medium the amplitude of the e.p.c. clamped at hyperpolarized

TABLE 1. Comparison of amplitude of e.p.c. hyperpolarized by short (15 msec) and long pulses (longer than 10 sec)

End-plate	Amplitude of e.p.c. hyperpolarized by: Short pulse ($\times 10^7$ A)	Amplitude of e.p.c. hyperpolarized by: Long pulse ($\times 10^7$ A)	Hyperpolarization (mV)	Ratio of amplitudes (%)	K^+ (mM)	Mg^{2+} (mM)
		A. High-potassium solution				
a	1·40	1·15	66	82	18·8	10
b	2·52	2·06	66	82	6·0	10
b	2·39	1·81	66	76	12·0	10
c	2·18	1·93	54	88·5	6·0	10
c	2·40	1·97	54	81	6·0	10
d	1·40	1·17	53	82·5	4·0	3·5
e	3·04	2·68	37	88·5	7·0	0
e	3·25	2·81	48	86·5	7·0	0
		B. Normal solution				
f	1·45	1·46	70	101	2·0	0
g	3·20	3·40	39	106	2·0	0
h	0·87	1·07	65	123	2·0	0
i	1·21	1·22	61	100	2·0	0
j	0·78	0·76	53	97·5	2·0	0

TABLE 2. Effect of potassium concentration on mean quantum content (m) of e.p.p. in Ringer's solution containing (mM) Mg 25, Ca 0·8

End-plate	1·5 mM-K	10 mM-K	1·5 mM-K	10 mM-K
a	0·76	1·32	0·74	—
b	0·32	0·45	0·17	0·38

Quantum content (m) was calculated from the relation $m = \log_e$ (number of impulses/number of failures) (del Castillo & Katz, 1954*a*). Each value was calculated from 160 to 200 impulses.

potentials by short pulses was larger than that clamped with long pulses, although no striking difference could be detected in normal solution (Table 1*B*).

In order to clarify the reason for this result, the quantum content was measured with an end-plate in high magnesium and low calcium, following which the potassium concentration was increased with the same concentration of magnesium and calcium. Table 2 shows that in high-potassium medium the quantum content increased. The result in the preceding section suggested that the change in potassium concentration took place rather slowly after the hyperpolarization of membrane. Thus the difference in

amplitude of e.p.c. with the membrane hyperpolarized by short or by long pulses may be interpreted as follows: when the membrane was hyperpolarized by short pulses there may have been little change in potassium concentration near the muscle membrane, and the quantum content of e.p.c. was rather large, but when the membrane was hyperpolarized with long pulses a change in potassium concentration may have occurred and

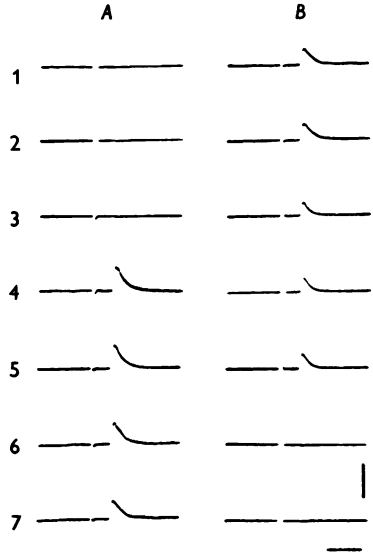

Fig. 5. E.p.c. evoked every 3 sec in 12 mM-K^+ solution. Between $A2$ and $A3$ membrane was suddenly hyperpolarized (61 mV), and e.p.c. appeared in $A4$. $B1$ and $B2$ were recorded during hyperpolarization; between $B2$ and $B3$ membrane potential was returned to resting value and neuromuscular transmission was blocked after $B6$. Current scale, 2×10^{-7} A; time scale, 5 msec.

the quantum content may have decreased. It is known that KCl relieves the curare block at the neuromuscular junction (Wilson & Wright, 1937; Brown & von Euler, 1938; Walker & Laporte, 1947) and the increase in quantum content in high-potassium solution may be one of the reasons for the decurarizing action of potassium ion.

In some cases, when 7·5 mM-K^+ Ringer's solution was introduced neuromuscular transmission was blocked and no potential change could be obtained by applying supermaximal stimuli to the nerve. In such instances hyperpolarization of muscle membrane restored neuromuscular transmission in a few seconds. Figure 5 shows e.p.c. evoked every 3 sec. Between $A2$ and $A3$ the membrane potential was suddenly hyperpolarized by 61 mV. The e.p.c. appeared in $A4$ and thereafter transmission continued. $B1$ and $B2$ are e.p.c.s obtained during the hyperpolarization and between $B2$ and $B3$ the membrane was returned to its resting potential.

Transmission continued for about 10 sec after the cessation of inward current and then was blocked suddenly. The time course of the recovery of transmission by current flow was similar to that of the change in frequency of m.e.p.p. in high potassium medium. This sudden block of transmission may occur around the arborizations of nerve fibres, suggesting that the change in concentration of potassium ion was not confined to the nerve terminals or the groove between nerve terminal and the end-plate membrane, but also occurred in a wider area around the nerve arborization.

Effect of current on the post-tetanic increase in frequency of miniature discharge

Various investigators have hypothesized that post-tetanic potentiation can be explained in terms of an increased external potassium ion concentration (cf. Hughes, 1958). Recent observations have shown that after a tetanus frequency of the m.e.p.p. is increased (Liley, 1956*b*; Brooks,1956; Hubbard, 1959). Since this post-tetanic potentiation of discharge frequency runs a time course paralleling the post-tetanic potentiation of

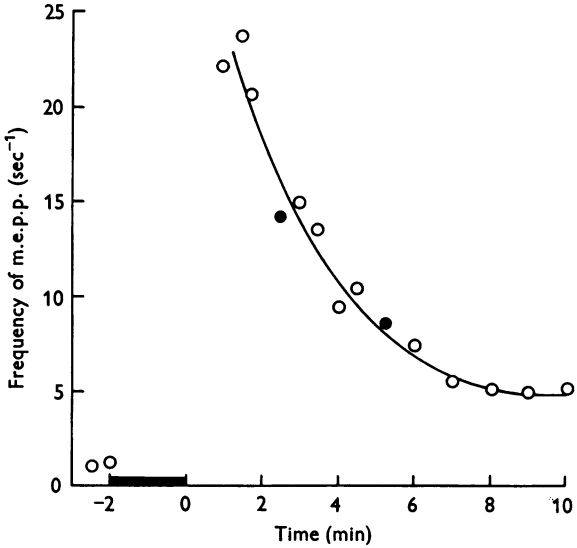

Fig. 6. Effect of current on post-tetanic increase of miniature discharge frequency. Each circle represents mean frequency for about 10 sec. ○, without current; ●, during passing inward current, hyperpolarizing the membrane about 50 mV. During thick bar tetanic stimulus (10/sec) was applied.

response, there is good reason for postulating a common mechanism. The increase in miniature discharge frequency might also be explained in terms of an increased external potassium ion concentration. This hypothesis was tested by passing inward current through the muscle membrane during post-tetanic potentiation of discharge frequency. If the increase in dis-

charge frequency were due to an increased potassium concentration around nerve terminals, inward current would be expected to reduce the potassium concentration near the muscle membrane and the discharge frequency might decrease during and after passing current. In Fig. 6 the changes in mean frequency of m.e.p.p.s in such an experiment are shown. During the thick bar a 10/sec tetanus was applied. After the tetanus the miniature frequency increased to more than 20 times its control value and then decayed gradually. Open circles show mean frequencies obtained without current, and the filled circles are those obtained when the membrane was hyperpolarized by about 50 mV. The result indicates that inward current did not change the time course of the recovery of miniature discharge frequency. This result is contrary to the above hypothesis, i.e. that the post-tetanic potentiation of miniature discharge is due to the increased potassium concentration around nerve terminals.

DISCUSSION

The present results suggest that the passage of electric current through the muscle produces an appreciable change in concentration of potassium ion near the muscle membrane, at least at the end-plate. Since, if ion mobilities were uniform as in free solution, concentration changes would not occur by passing current, the change near the muscle may be due to a selective permeability of the resting muscle membrane. The resting muscle is permeable to potassium and chloride ions, and inward current through the membrane may consist mainly of inward-moving potassium ions and outward-moving chloride ions. If mobilities and diffusion constants of ions are uniform everywhere, other than in the membrane, potassium ions carried through the membrane will be replaced by ions brought to the membrane by diffusion and by current. If the stretch of end-plate is assumed to be small compared with the characteristic length of muscle fibre, the current density may be considered as uniform throughout the end-plate region. If the end-plate is assumed to be on the exposed surface of the muscle fibre, the change in concentration near the end-plate can be represented approximately as a one-dimensional diffusion problem. The potassium concentration C at time t and at distance x normal to the membrane is a solution of the following equation:

$$D\frac{\partial^2 C}{\partial x^2} = \frac{\partial C}{\partial t},$$

$$C = C_0 \quad (t = 0),$$

$$D\frac{\partial C}{\partial x} = \frac{Vg_K}{F} - p \quad (x = 0),$$

$$C \neq \infty \quad (x = \infty).$$

The amount of potassium ion carried by current through unit area of membrane in unit time is Vg_K/F, where V is potential change produced by current through muscle membrane (outside potential minus inside potential). F is the Faraday constant, and g_K the potassium conductance per unit area of membrane and D the diffusion constant. p is the amount of potassium ion carried by current to a unit area of membrane per second and a function of the concentration and the potential gradient in solution. In the present conditions this amount may be negligibly small compared to Vg_K/F, because the potassium concentration in solution is much less than that of other ions. g_K is a function of potassium concentration and it is assumed that $g_K = aC$, where a is a constant. When the muscle membrane potential is near the potassium equilibrium potential, g_K is approximately

$$P_K \frac{F^3 V_m}{(RT)^2} K_o \left(1 - \frac{K_o}{K_i}\right)^{-1},$$

where V_m is membrane potential (outside potential minus inside potential) and K_o and K_i are outside and inside concentrations of potassium ion respectively. If V_m and P_K are assumed to be constant and K_o is much smaller than K_i, g_K may be considered directly proportional to C. The solution of the diffusion equation with these initial and boundary conditions can be easily obtained by Laplace transformation (cf. Crank, 1956), and

$$C = C_0 \left[\operatorname{erf}\left(\frac{x}{2\sqrt{Dt}}\right) + e^{hx+Dth^2} \left\{1 - \operatorname{erf}\left(\frac{x}{2\sqrt{Dt}} + h\sqrt{Dt}\right)\right\}\right].$$

At $x = 0$ the solution is reduced to

$$C = C_0 e^{Dth^2}\{1 - \operatorname{erf}(h\sqrt{Dt})\},$$

where

$$\operatorname{erf}(Z) = \frac{2}{\sqrt{\pi}} \int_0^Z e^{-u^2} du \quad \text{and} \quad h = \frac{Va}{DF} \quad (p \text{ is neglected}).$$

If the current which changes the membrane potential by 50 mV in 16·7 mM-K^+ changes the potassium concentration near the muscle membrane by 2 mM in 10 sec and g_K is assumed to be about 500 μmho/cm^2 at $C = 10$ mM (cf. Hodgkin & Horowicz, 1959), the diffusion constant should be about $4{\cdot}5 \times 10^{-7}$ cm^2/sec. This value is much smaller than that of KCl in aqueous solution (in the order of $1{\cdot}6 \times 10^{-5}$ cm^2/sec). The calculation is merely an approximation but suggests that some structures might act as diffusion barriers.

The fact that the inward current had no influence on the post-tetanic increase in discharge frequency seems to exclude the possibility that the post-tetanic frequency change is due to an increase in potassium concentration around nerve terminals. Recent observation shows that post-

tetanic potentiation of synaptic transmission can be explained by an increase in the membrane potential and amplitude of action potential of presynaptic fibres (Lloyd, 1949; Eccles & Krnjević, 1959). However, it seems difficult to explain the post-tetanic increase of miniature discharge in terms of increased membrane potential and its mechanism remains obscure.

SUMMARY

1. The effect of inward current through the muscle membrane on the potassium concentration near muscle membrane was investigated by using the frequency of miniature end-plate potentials (m.e.p.p.) as an index of potassium concentration.

2. The frequency of spontaneous m.e.p.p. was markedly increased in concentrations of potassium greater than about 5 mM, smaller changes being observed in lower concentrations.

3. The frequency of miniature discharge in high-potassium medium was decreased by passing inward current through the muscle membrane at end-plate region. After the cessation of current the frequency returned to original level.

4. The change in frequency of miniature discharge after the start and the cessation of inward current was rather slow and 5–10 sec elapsed before the frequency reached fairly stationary level.

5. These results can be explained by a decrease in potassium concentration around nerve terminals during passage of inward current.

6. Inward current relieved the block of neuromuscular transmission occurring in high-potassium medium. The amplitude of end-plate current during hyperpolarization by short pulses was larger than that during long pulses. These results can also be explained in terms of changes in potassium concentration.

7. Inward current had no influence on the post-tetanic increase of miniature discharge frequency, which excludes the possibility that the post-tetanic increase of miniature discharge frequency is caused by increase in external potassium concentration around nerve terminals.

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