J. Physiol. (1957) 139, 15–26

AN ANALYSIS OF JUNCTIONAL POTENTIALS OF INTRAFUSAL MUSCLE FIBRES IN FROGS

By K. KOKETSU and S. NISHI

From the Division of Psychiatry, Neuropsychiatric Institute, University of Illinois College of Medicine, Chicago, Illinois, U.S.A. and Department of Physiology, Kurume University, School of Medicine, Kurume, Japan

(*Received* 27 *March* 1957)

In the organization of the frog's skeletal musculature three different types of fibres may be recognized: twitch (extrafusal), slow and intrafusal. In each kind of fibre junctional potentials of characteristic configuration may be produced by motor nerve volleys. At the neuromuscular junction of twitch fibres, the end-plate potential (e.p.p.) is found. This has been analysed in detail by Fatt & Katz (1951), who showed that the active phase of neuromuscular transmission is a brief impulsive event lasting only a few msec and that the decline of e.p.p. is determined by the electrical properties of the resting muscle membrane. According to Fatt & Katz (1951) and del Castillo & Katz (1954), the action of the neuromuscular transmitter produces a relatively non-selective increase of ion permeability which drives the junctional membrane toward an equilibrium potential near zero membrane potential. In slow skeletal muscle fibres small nerve junctional potentials (s.j.p.'s) have been described by Kuffler & Vaughan Williams (1953). Their time course is slower than that of the e.p.p. and their decay shows a phase of hyperpolarization. According to Burke & Ginsborg (1956*a*) the slower time course of the s.j.p. is due largely to the multiple innervation of the slow muscle fibres by the small motor nerve fibres, and the diphasic decay may be ascribed to 'delayed rectification' occurring in the depolarized membrane. Burke & Ginsborg (1956*b*) have also shown that the action of the transmitter on slow muscle fibres is similar to that on twitch muscle fibres; the transmitter reduces the resistance of the junctional membrane and drives the membrane potential toward a new equilibrium potential.

The junctional potentials of intrafusal muscle fibres (i.j.p.'s) have been described by Koketsu & Nishi (1957) who found that the intrafusal muscle

fibres receive multiple innervation from motor nerve fibres and that the time course of their junctional potentials is relatively fast and shows no appreciable diphasic decay. In the present paper the membrane characteristics of the intrafusal muscle fibres are studied and the process establishing the i.j.p. is analysed. Experiments on twitch and slow muscle fibres have also been made, so that the properties of the junctional potentials in different kinds of fibres may be compared.

METHODS

Preparations. The species *Rana nigromaculata* was used. Isolated single muscle spindles of M. extensor longus dig. IV were used for experiments on intrafusal muscle fibres. The isolation technique has been described in a previous paper (Koketsu & Nishi, 1957).

M. iliofibularis was used for experiments on twitch and slow muscle fibres. The slow muscle fibres are grouped together at the inner side of this muscle and several nerve twigs enter from this side. All these nerve twigs, except one thin twig which innervates the inner side of the muscle, were cut and stimuli were applied to the whole nerve trunk. Movement caused by muscle twitches was prevented by this procedure, presumably because most large motor nerve fibres were cut. Micro-electrodes were inserted from the inner surface of the muscle. About 10–20% of the fibres in this part of the muscle seemed to be slow muscle fibres that could be distinguished from twitch muscle fibres by their resting potentials and by the presence of s.j.p.'s (cf. Kuffler & Vaughan Williams, 1953).

Micro-electrodes. A single intracellular micro-electrode of low resistance was used both for recording potentials and applying currents, the potential drop across the electrode being balanced by a Wheatstone-bridge circuit (Koketsu & Nishi, 1957). When the resistance of micro-electrodes was between 15 and 20 MΩ, steady currents of less than 4×10^{-9} A could be passed through the electrodes. The effective resistances of twitch and slow muscle membranes were lower than those of intrafusal muscle fibres. Consequently, stronger currents had to be applied to these muscle fibres, particularly to the twitch muscle fibres. Micro-electrodes of a resistance less than 10 MΩ with tip diameter less than 1 μ were selected for the twitch and slow muscle fibres. When the resistance of the micro-electrode was about 5 MΩ, steady currents of 5×10^{-8} A could be applied without any technical difficulty.

Solution and temperature. Ringer's solution of the following composition was used: 112 mM-NaCl, 2·0 mM-KCl, 1·8 mM-$CaCl_2$, 2·4 mM-$NaHCO_3$. All experiments were conducted at room temperature (20°–30 C) in summer.

RESULTS

Effective membrane resistance and rectification

Applying the cable theory of Hodgkin & Rushton (1946) to the case of a single intracellular micro-electrode used both for recording potentials and applying currents, it may be shown that the electrotonic potential V is related to the applied steady current I by the following equation

$$V = I/2\sqrt{(r_m r_i)},$$

where r_m and r_i are, respectively, the transverse resistance of the membrane times unit length, and the longitudinal resistance of the fibre per unit length. The term $1/2\sqrt{(r_m r_i)}$ is the effective resistance of the muscle membrane.

A study was made of the relation between the voltage and current on intrafusal, twitch and slow muscle fibres under identical experimental conditions.

When the displacement of potential was less than 10 mV in either direction, no appreciable rectification was observed in any of the three kinds of muscle fibre. The effective resistance of each fibre could therefore be calculated from the slope of the voltage-current relationship. The results obtained are given in Table 1. It will be seen that there was a distinct difference in the effective resistance between the three types of muscle fibre, although there was some variance within each group. This result has been represented diagrammatically in Fig. 1, which shows voltage-current relationships drawn at slopes corresponding to the mean effective resistance of each kind of fibre.

In many intrafusal muscle preparations spike potentials cannot be recorded, even when the membrane is depolarized considerably (Koketsu & Nishi, 1957),

TABLE 1. Effective resistances (kΩ) derived from voltage-current relationship. The parenthesized values indicate the resting potential (mV)

Fibre	Intrafusal muscle fibres	Twitch muscle fibres	Slow muscle fibres
I	4900 (38)	310 (90)	1500 (58)
II	5800 (40)	290 (87)	1000 (38)
III	4600 (38)	260 (85)	1200 (45)
IV	4600 (35)	320 (92)	1300 (53)
V	5400 (45)	330 (90)	900 (48)
VI	5600 (43)	300 (88)	1400 (55)
VII	5000 (38)	—	—
VIII	4500 (42)	—	—
Mean value	5050 (40)	300 (89)	1200 (50)

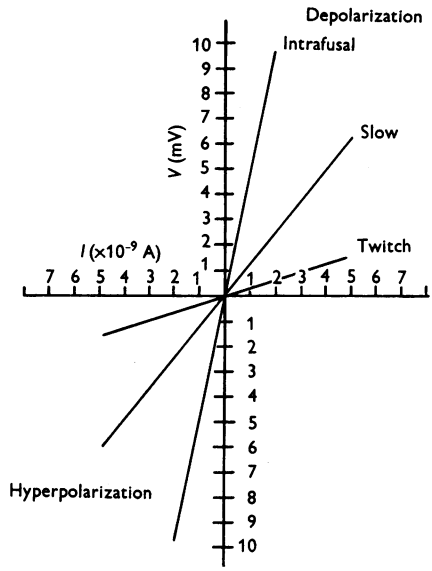

Fig. 1. Voltage/current relationships for intrafusal, slow and twitch muscle fibres. Each straight line represents the average slope obtained from eight intrafusal, six slow and six twitch muscle fibres. Abscissa (*I*), current strength in 10^{-9} A; ordinate (*V*), displacement from the resting potential in mV.

and it is then possible to study the voltage-current relation over a wider range. Fig. 2 shows electrotonic potentials obtained from such a preparation. As mentioned above, no changes of effective resistance occur during the passage of a weak steady current. When, however, the membrane is depolarized by more than 10 mV, V decays gradually during the passage of the current. This rectification becomes clearly apparent when the applied current is strong, and the initial part of the catelectrotonic potential then shows a hump. In the case of hyperpolarization, when V exceeds approximately 20 mV, the anelectrotonic potential increases gradually during the passage of currents. Thus, the intrafusal muscle membrane behaves as a rectifier, having a greater resistance to anodic currents than to cathodic currents.

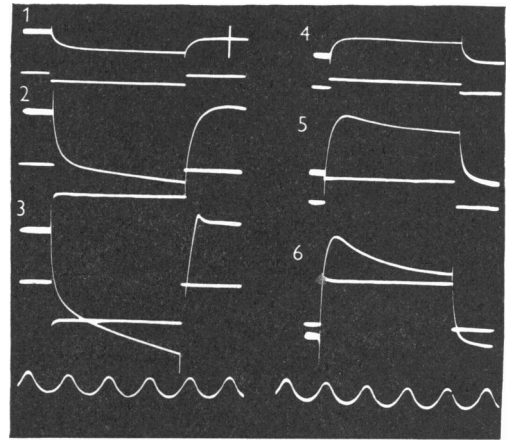

Fig. 2. Electrotonic potentials (upper traces in each record) produced by anodic (left-hand column) and cathodic (right-hand column) currents. Depolarization upwards. The relative strength of applied currents is indicated by lower traces in each record. Resting potential, 35 mV (note a local response in record 3); time mark, 60 cycles; vertical line, 10 mV.

In twitch muscle fibres, local responses and spike potentials soon appear when depolarization exceeds about 30 mV. To avoid this complication, sodium ion in Ringer's solution was replaced by magnesium ion or sucrose and electrotonic potentials of twitch muscle fibres were recorded, often with the use of two micro-electrodes in one fibre (cf. Fatt & Katz, 1951). Numerous experiments indicated that the twitch muscle fibre membrane also has a rectifying property for applied steady currents, although this could not be observed in fibres which were seriously injured. Rectification usually appeared when the membrane was depolarized by more than 20–25 mV.

That the slow muscle fibre membrane behaves as a rectifier has been shown by Burke & Ginsborg (1956*a*); in the present experiment, a reduction in resistance was usually observed when depolarization exceeded about 10 mV.

Burke & Ginsborg (1956*a*) suggested that, as in the squid axon (Cole & Curtis, 1941; Hodgkin, Huxley & Katz, 1949; Hodgkin & Huxley, 1952*a*), rectification in the slow muscle fibres is due to changes in permeability of the membrane to potassium ion which occur with a delay. It seems likely that the rectification observed in intrafusal and twitch muscle fibres can also be explained by such a delayed permeability change. It is possible, however, that in the case of intrafusal muscle fibres a transient increase of sodium ion permeability may also contribute to the initial hump on the catelectrotonic potential; for although propagated spike potentials are not always produced in these fibres they are capable of giving local responses (see below).

A phase of hyperpolarization following the catelectrotonic potential is observed in Fig. 2 (Records 5 and 6). Such a hyperpolarization was usually observed in intrafusal muscle fibres as well as in twitch and slow muscle fibres. In record 2 of Fig. 2 the anelectrotonic potential decays with a phase of depolarization. These phenomena may also be explained by delayed rectification (cf. Burke & Ginsborg, 1956*a*).

As seen in record 3 of Fig. 2, when strong hyperpolarization was applied, a small local potential was induced on the subsequent depolarization. When stronger hyperpolarization was applied, spike potentials were produced by the off-effect of hyperpolarization. As mentioned in a previous paper (Koketsu & Nishi, 1957), spike potentials were easily produced in intrafusal muscle fibres by the off-effect of hyperpolarization, even when none was produced by strong depolarization. This may be due to the removal of inactivation of a 'sodium-carrier' mechanism by strong hyperpolarization (Hodgkin, 1951; Hodgkin & Huxley, 1952*b*).

Time course of junctional and electrotonic potentials

In several experiments on intrafusal, twitch and slow muscle fibres the electrode used for polarizing and recording electrotonic potentials was also employed to record the junctional potential set up by a nerve stimulation at the same point of the muscle fibre. A comparison of the time course of the two potentials could then be made. As shown in Fig. 3, it was found that in twitch muscle fibres the decay of a locally recorded e.p.p. was always much faster than that of the electrotonic potential. In slow muscle fibres, on the other hand, the decay of the s.j.p. was always much slower than that of the electrotonic potential. This may be explained by the fact that most twitch muscle fibres have only one junctional region, while slow muscle fibres are densely innervated so that their whole surface is depolarized on nerve stimulation (Burke & Ginsborg, 1956*a*). In intrafusal muscle fibres there was comparatively little difference between the time course of decay of the junctional potential and that of the electrotonic potential. This suggests that these fibres occupy, as regards distribution of nerve endings, an intermediate position, i.e., while

innervated over most of their length, the density of the innervation may be less than in slow muscle fibres.

The i.j.p. sometimes shows a diphasic decay, i.e. it is followed by a phase of hyperpolarization, which may be due to membrane injury caused by insertion of the micro-electrode and consequent loss of membrane potential (Koketsu & Nishi, 1957). According to Burke & Ginsborg (1956*a*), the diphasic decay of s.j.p. is due to delayed rectification of depolarized membrane. It is conceivable that the hyperpolarizing phase of the i.j.p. occurs in the same way.

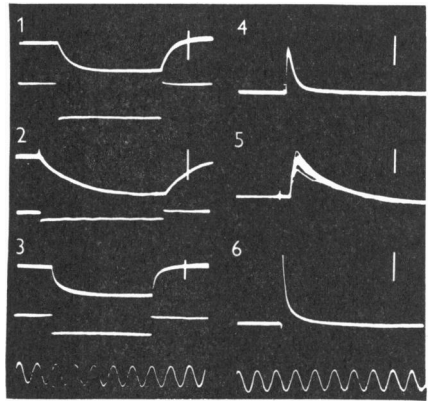

Fig. 3. Electrotonic potentials (left-hand column) and junctional potentials (right-hand column). Records 1, 2 and 3 are electrotonic potentials of intrafusal, slow and twitch muscle fibres, respectively. Records 4, 5 and 6 are i.j.p., s.j.p. and e.p.p., respectively. The electrotonic and junctional potentials were recorded from the same position of each muscle fibre. Lower traces in each record of left-hand column indicate strength of applied currents: record 1 ($1{\cdot}2 \times 10^{-9}$ A), 2 ($5{\cdot}5 \times 10^{-9}$ A) and 3 ($2{\cdot}7 \times 10^{-8}$ A); time mark, 60 cycles; vertical lines, 5 mV.

Membrane constants

If the specific internal resistance (R_i) and the radius (ρ) of the intrafusal muscle fibres are known, their membrane constants can be calculated from the values of the effective resistance ($1/2\sqrt{(r_m r_i)}$) and the time constant (τ_m) of the resting membrane. R_i was taken as 250 Ω cm, on an assumption that the internal conductivity of intrafusal muscle fibre is the same as that of the twitch muscle fibre (Fatt & Katz, 1951). The diameter of intrafusal muscle fibres was measured directly from fresh preparations; the mean value was 15 μ. The length of the intrafusal muscle fibres was approximately 5 mm, and the position of inserted micro-electrodes was about 1·5 mm from the equatorial region of the muscle spindle (Koketsu & Nishi, 1957).

The value of the specific membrane resistance (R_m), obtained from the effective resistance (5000 kΩ cm), R_i (250 Ω cm) and ρ (7·5 μ), is 3300 Ω cm^2. Then, the length constant (λ) was calculated as 0·72 mm.

The approximate value of the time constant of resting membrane can be

obtained from the electrotonic potential (Hodgkin & Rushton, 1946). In the present experiment, a single intracellular micro-electrode was used both for recording potentials and applying currents; therefore, the time course of the electrotonic potential V can be expressed as follows

$$V = V_o \operatorname{erfc} \sqrt{\frac{t}{\tau_m}},$$

where V_o and τ_m are, respectively, the final values of electrotonic potentials and the time constant of the resting muscle membrane. Thus, the time constant of resting membrane will be the time the electrotonic potential takes to decay to 16% of its initial value. The mean value of the time constant obtained from the fibres used in Fig. 1 was 19 msec. Then the capacitance of the membrane was calculated as 5·5 μF/cm^2.

TABLE 2. Membrane constants of intrafusal, twitch and slow muscle fibres. R_i of the three different kinds of muscle fibres is assumed to be 250 Ω cm

	Intrafusal	Twitch	Slow
$1/2\sqrt{(r_m r_i)}$ (kΩ)	5000	300	1200
$2 \times \rho$ (μ)	15	100	44
R_i (Ω cm)	250	250	250
R_m (Ω cm^2)	3300	3600	4900
λ (mm)	0·72	1·9	1·47
τ_m (msec)	19	16	45
C_m (μF/cm^2)	5·5	4·5	9·2

Although these calculated values may differ to some extent from the true membrane constants of intrafusal muscle fibres, it seemed interesting to compare them with membrane constants of twitch and slow muscle fibres obtained by identical experimental and calculating procedures. The specific internal resistance of both twitch and slow muscle fibres was taken as 250 Ω cm. An attempt to distinguish between these two kinds of muscle fibres had, however, to be made for the purpose of measuring their diameters. Twenty large and twenty small muscle fibres were therefore isolated from the inner side of fresh iliofibularis preparations and tested to see whether they gave action potentials on direct electrical stimulations. It was found that the large muscle fibres always produced action potentials; most of the small muscle fibres, on the other hand, did not give action potentials and it was therefore assumed that they were slow muscle fibres. The mean diameters of each group of fibres and the membrane constants calculated on this basis are given in Table 2.

Action of neuromuscular transmitter

The action of the neuromuscular transmitter (acetylcholine) on the twitch muscle membrane is brief and impulsive, lasting only a few msec, and it produces a relatively non-selective increase in the ionic permeability of the end-plate membrane (Fatt & Katz, 1951). Consequently, the resting potential of

the end-plate membrane is driven toward an equilibrium potential (del Castillo & Katz, 1954). Burke & Ginsborg (1956*b*) demonstrated that the s.j.p. is annulled when the membrane is depolarized to a particular level—the 'reversal level'. This finding shows that the action of the neuromuscular transmitter on the slow muscle membrane drives the resting potential of the junctional membrane toward a new equilibrium potential. To determine whether the action of the neuromuscular transmitter on the intrafusal muscle membrane is of similar nature, the effect of displacement of the resting

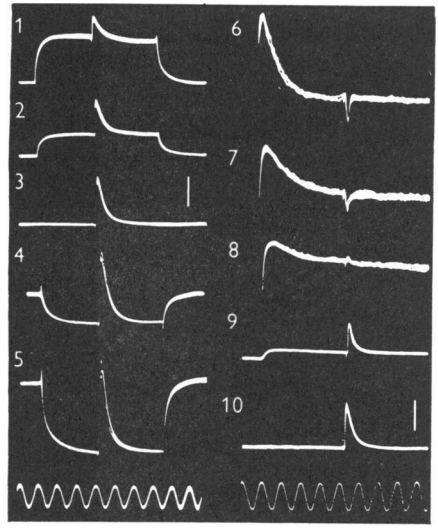

Fig. 4. The effect of changing the membrane potential on the i.j.p. The resting potential is altered by passing rectangular currents through the membrane. Depolarization upwards. In the left column, i.j.p.'s are superimposed on electrotonic potentials produced by rectangular current pulses; record 3 shows the i.j.p. alone. The records on the right were obtained from another preparation. The i.j.p. is superimposed on catelectrotonic potential produced by long-lasting rectangular current pulses. In records 6, 7 and 8, balancing of the Wheatstone bridge circuit was not satisfactory and base lines were not recorded. Record 10 is of the i.j.p. alone. Time mark, 60 cycles; vertical lines, 5 mV.

potential on the i.j.p. was studied. It was found that when the membrane is hyperpolarized, the amplitude of the i.j.p. becomes higher; when it is depolarized, the i.j.p. becomes smaller than normal size. If the depolarization of the membrane exceeds a certain level, the i.j.p. is reversed (see records of right-hand column of Fig. 4). Unfortunately, the exact relation between the amplitude of the i.j.p. and the membrane potential could not be obtained in the present experiment, because the resistance of the micro-electrodes often changed when strong outward currents were applied, so that the exact value of the membrane potential could not always be measured. However, the changes in amplitude and sign of the i.j.p. strongly indicate that it represents

a displacement of the membrane potential toward a new equilibrium potential, and it may therefore be concluded that the action of the neuromuscular transmitter on the intrafusal muscle fibre membrane is similar to that on the twitch and slow muscle fibre membrane.

Local potential

It has been reported (Koketsu & Nishi, 1957) that many intrafusal muscle preparations do not produce spike potentials but give a junctional potential which shows a long-lasting slow potential superimposed on the falling phase of i.j.p. (Fig. 5). These slow potentials show summation on application of two successive nerve stimuli and disappear on tetanic stimulation (see records 13 and 14 of Fig. 5). Insertion of micro-electrodes will easily injure the thin intrafusal muscle membrane and abolish spike potentials. It was suggested

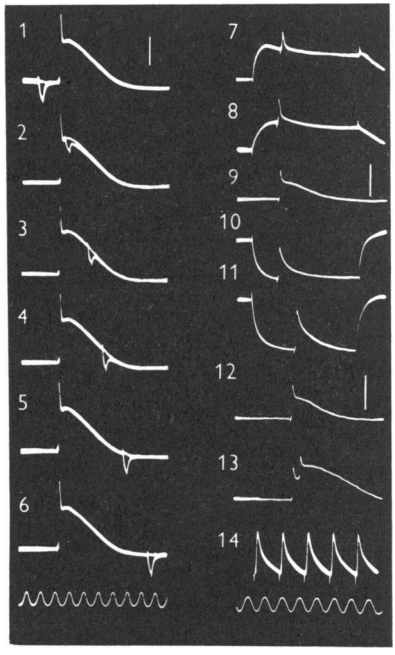

Fig. 5. Characteristics of the slow potential superimposed on the falling phase of i.j.p. In records of left column, short test pulses were applied through membrane during the course of the slow potential: the size of test pulses in record 1 represents the conductivity of resting membrane. Records 7–11 were obtained from another preparation and demonstrate the effect of changing the membrane potential on the slow potential. Record 9 shows the junctional potential alone. Depolarization upwards. Records 12 and 14 were obtained from another preparation and demonstrate the summation of the slow potential and the effect of tetanic stimulation on the slow potential. Time mark, 60 cycles; vertical lines, 5 mV (left column), 10 mV (right column).

in the preceding paper (Koketsu & Nishi, 1957) that the slow potential superimposed on the i.j.p. may be a local response evoked only from injured muscle fibres. Nevertheless, it seemed interesting to investigate the characteristics of these slow potentials and to study how they are established.

If the slow potential is a potential change caused by changes in permeability towards diffusible ions, the conductivity of the muscle membrane must be increased during its course. The conductivity of muscle membrane during the development of slow potential was therefore measured by applying short test pulses to the membrane through an intracellular micro-electrode. Records 1–6 of Fig. 5 show such an experiment. The size of test pulses is reduced during the course of the slow potential, particularly during its initial part. This indicates that the conductivity of membrane is increased in the initial part of slow potential and gradually returns to normal value as the membrane is repolarized. The slow potential was always followed by a phase of slight hyperpolarization, but there was no detectable increase in conductivity in the course of this hyperpolarization. The slow potential simply disappeared when the membrane was depolarized to a level near the peak of the slow potential. With hyperpolarization, the falling phase of i.j.p. became slower and the slow potential disappeared.

DISCUSSION

The time course of the falling phase of i.j.p. is determined by the time constant of resting membrane, the density of the multiple innervation, and the length constant (λ) of muscle membrane. Comparison of the time course of junctional and electrotonic potentials of intrafusal and slow muscle fibres suggests that the density of the junctional region of the intrafusal muscle fibre is less than that of the slow muscle fibre. However, it is essential to note that the length constant (λ) of slow muscle fibres is larger compared with that of intrafusal muscle fibres. An alternative explanation for the slower time course of s.j.p. is that the falling phase of s.j.p. may not be a simple passive decay and that some active transport of ions takes place in the course of its falling phase.

In the course of the present experiment, s.j.p.'s which showed no diphasic decay were frequently observed, particularly immediately after insertion of micro-electrodes. It was also observed that the after-positivity of s.j.p.'s, as of i.j.p.'s, may be produced from injured membrane. Burke & Ginsborg (1956*a*), suggested that the after-positivity of the s.j.p. may be due to delayed rectification, which occurs in depolarized membrane. However, it is conceivable that the after-positivity is due to the injured muscle membrane.

When the i.j.p. reaches a certain potential, local responses will be induced from neighbouring muscle membrane. Such an active local potential is a pre-process of a sudden and specific increase of sodium ion (Hodgkin, 1951). A low membrane potential of muscle or nerve fibres causes inactivation of the

'sodium carrier' mechanism. If the activation of the 'sodium carrier' mechanism is weak, the local potential may remain without producing a spike potential. In twitch muscle fibres the spike potential was seen to disappear and the slow local potential remained when muscle fibres were injured by repeated insertion of micro-electrodes. The same situation must occur even more easily in thin intrafusal muscle fibres. The slow potential which was frequently superimposed on the falling phase of i.j.p. is explained as a local potential produced from injured membrane close to the junctional region.

SUMMARY

1. The effective membrane resistance and rectifying properties of intrafusal muscle fibres have been studied and compared with those of twitch and slow muscle fibres. In most experiments, a single intracellular electrode was used for polarizing and recording.

2. The time courses of junctional and electrotonic potentials of intrafusal muscle fibres have been compared with those of twitch and slow muscle fibres. It seems likely that the multiple innervation of intrafusal muscle fibres is less dense than that of slow muscle fibres.

3. The membrane constants of intrafusal, twitch, and slow muscle fibres have been calculated.

4. The action of the neuromuscular transmitter on the intrafusal muscle membrane appears to be identical with its action on the twitch and slow muscle membrane.

5. The falling phase of i.j.p. is a passive decay, its time course determined by the time constant of resting membrane and multiple junctional regions of muscle membrane. A slow potential which was frequently superimposed on the falling phase of i.j.p. is interpreted as a local potential produced from injured membrane close to the junctional region.

This research was supported jointly by U.S.P.H.S. Grant B-413C 3 and the Teagle Foundation.

REFERENCES

BURKE, W. & GINSBORG, B. L. (1956*a*). The electrical properties of the slow muscle fibre membrane. *J. Physiol.* **132**, 586–598.

BURKE, W. & GINSBORG, B. L. (1956*b*). The action of the neuromuscular transmitter on the slow fibre membrane. *J. Physiol.* **132**, 599–610.

COLE, K. S. & CURTIS, H. J. (1941). Membrane potential of the squid giant axon during current flow. *J. gen. Physiol.* **24**, 551–563.

DEL CASTILLO, J. & KATZ, B. (1954). The membrane change produced by the neuromuscular transmitter. *J. Physiol.* **125**, 546–565.

FATT, P. & KATZ, B. (1951). An analysis of the end-plate potential recorded with an intracellular electrode. *J. Physiol.* **115**, 320–369.

HODGKIN, A. L. (1951). The ionic basis of electrical activity in nerve and muscle. *Biol. Rev.* **26**, 339–409.

HODGKIN, A. L. & HUXLEY, A. F. (1952*a*). Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *J. Physiol.* **116**, 449–472.

HODGKIN, A. L. & HUXLEY, A. F. (1952*b*). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117**, 500–544.

HODGKIN, A. L., HUXLEY, A. F. & KATZ, B. (1949). Ionic currents underlying activity in the giant axon of the squid. *Arch. Sci. physiol.* **3**, 129–150.

HODGKIN, A. L. & RUSHTON, W. A. H. (1946). The electrical constants of a crustacean nerve fibre. *Proc. Roy. Soc.* B, **133**, 444–479.

KOKETSU, K. & NISHI, S. (1957). Action potentials of single intrafusal muscle fibres of frogs. *J. Physiol.* **137**, 193–209.

KUFFLER, S. W. & VAUGHAN WILLIAMS, E. M. (1953). Small-nerve junctional potentials. The distribution of small motor nerves to frog skeletal muscle, and the membrane characteristics of the fibres they innervate. *J. Physiol.* **121**, 289–317.