EFFECTS OF POLARIZING CURRENTS ON LONG LASTING DISCHARGES IN THE FROG MUSCLE SPINDLE

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Many investigators\(^1,^{15,16}\) have observed that depolarizing currents applied to the terminal ramifications of spindle receptors elicit a slowly adapting discharge similar to that produced by a generator current during muscle stretch. However, there is a report\(^2\) demonstrating that adaptation occurs in the membrane process which converts generator currents into a sequence of action potentials. The present study was undertaken to identify the site of origin of the long-lasting discharges in the frog muscle spindle. As will be shown, the terminal ramification of the spindle axon possesses an ability to produce long-lasting discharges, but the parent axon, proximal from the capsule by more than 0.5 mm, does not. A preliminary report of the work has appeared elsewhere\(^9\).

METHODS

The experiments were carried out on thirty six spindles isolated from sartorius muscles of frogs (\textit{Rana nigromaculata}) at room temperature, which varied from 22 to 25\(^\circ\)C. The single parent axon of a spindle receptor was isolated along its intramuscular course until the capsule of the spindle receptor was cleared, but its capsule and its intrafusal muscle bundle were left attached to the remaining musculature.

The excised preparation was placed in a Ringer pool (RA) in a perspex box, and the isolated nerve passed into another Ringer pool (RB) through a liquid paraffin pool of 2 mm length. The paraffin pool was situated in a slit of 1 mm at the center of a partition between the two Ringer pools. The paraffin gap method was described in detail in an earlier paper\(^7\). A pair of calomel electrodes were inserted into subsidiary Ringer pools, each of which was connected with RA and RB by means of two Ringer-agar bridges. The electrodes were placed into an arm of a bridge circuit\(^9\), through which potential differences between the Ringer pools of RA and RB were led and simultaneously polarizing currents could be applied to the preparation. Rectangular pulses of various intensities, with durations between 0.1 and 1.0 sec, were delivered from an isolation unit of a stimulator. The bridge circuit was balanced with a direct
current of subthreshold strength. The current applied to the electrodes for polarization of the sensory terminal was detected as a potential drop across a 10 KΩ resistor inserted between one of the electrodes and the input of current in the bridge.

Electrotonic potentials and responses of the muscle spindle were recorded on the first beam of an oscilloscope (CRO) through a cathode follower input of DC amplifier. The polarizing current was displayed on the second beam of the CRO through a differential input of a DC amplifier employed as a current detector. In all records negativity at the sensory nerve terminal (in RA) relative to the proximal portion of the axon (in RB) was displayed on CRO as an upward deflection.

The tendon at one end of the muscle was attached to an isometric myograph and the tendon at the other end to a lever of an electromagnetic stretcher (cf. Ito7). When the muscle was fixed at its in situ length which ranged from 26 to 32 mm, it was termed '+0 mm' initial length. The term '+4 mm' means that the muscle is lengthened by 4 mm from the initial length. Tension of the muscle was detected by a RCA 5734 tube in the myograph, and displayed on the second beam of the CRO. The muscle was stretched by 2 mm from its initial length at a rate of 7 mm/sec.

RESULTS

Facilitation and depression of spontaneous discharges during polarization

Fig. 1A shows spontaneous discharges from a spindle receptor at the in situ (+0 mm) length of the muscle without a polarizing current (middle trace in each record) and those during catelectrotonic (upper deflections) and anelectrotonic (lower deflections) polarizations of three different strengths (a, b, and c). In these cases, the paraffin gap was situated at a point along the axon 0.2 mm apart from the spindle capsule. The discharge appeared during application of a catelectrotonic current of 5×10^-8 A for 0.1 sec, but could not be found during an anelectrotonic polarization of the same intensity (Fig. 1Aa). The frequency of the spontaneous discharge was increased during catelectrotonus with a current larger than 9×10^-8 A, while discharges with inversed phase occurred in the case of anelectrotonus of more than 12×10^-8 A (Fig. 1Ab and c). It is assumed from the polarity of the spikes that they may be initiated at a point along the axon in the proximal Ringer pool (RB) and be conducted antidromically through the paraffin gap. The frequency of the antidromic discharge is high at the onset of an anelectrotonic current but declines quickly during the current. The threshold of the catelectrotonic current for increasing the frequency of the spontaneous discharges was usually lower than that of the anelectrotonic current for producing the antidromic discharge.

Spontaneous discharges from a spindle receptor during application of catelectrotonic currents of six different intensities, which were given across the paraffin gap situated at a distance of 0.5 mm from the spindle capsule, were superimposed in Fig. 1Ba. The discharge frequency was increased rapidly with a slight increase in the suprathreshold catelectrotonic current, but no further increase was observed with the current over 12×10^-8 A. Similar
FIG. 1. A. Spontaneous discharges (middle line in upper traces) from a spindle receptor at +0 mm initial length of the muscle, and modified discharges during application of catelectrotonic (upper deflections) and anelectrotonic currents (downward deflections) of various amounts (a, $0.5 \times 10^{-7}$ A; b, $0.9 \times 10^{-7}$ A; c, $1.2 \times 10^{-7}$ A), shown in the lower traces. The paraffin gap was situated at a distance of 0.2 mm from the spindle capsule. Calibration; 20 msec, 2 mV for upper traces and $2 \times 10^{-7}$ A for lower traces. B. Spontaneous discharges from another spindle receptor at the in situ length of the muscle during application of various amounts of catelectrotonic currents (upper traces in each record) which were passed across a paraffin gap situated at various distances from the spindle capsule (a, 0.5 mm; b, 2 mm; c, 10 mm). The applied current is shown in the lower traces. Calibration; 0.1 sec, 1.0 mV for upper traces and $1 \times 10^{-7}$ A for lower traces.

effects of catelectrotonic currents on the discharge frequency were also observed under conditions where the distance between the paraffin gap and the spindle capsule was 1 mm or 1.5 mm, although the rate of the frequency increase became slower with increases in the distance. When the gap-capsule distance was 2 mm, the discharge frequency increased quite slowly with catelectrotonic current under $7 \times 10^{-8}$ A, while it increased rapidly with current over $9 \times 10^{-8}$ A, as shown in Fig. 1Bb. Such a tendency became more prominent when the distance was increased to 3 mm or more (Fig. 1Bc).

FIG. 2 shows an acceleration of spontaneous discharge of a spindle receptor during a catelectrotonic current of $1 \times 10^{-7}$ A applied across a paraffin gap situated at 0.5 mm, 3 mm and 10 mm from the capsule. The discharge frequency was given by the reciprocal of the interval of spikes, and plotted
FIG. 2. Discharge frequency in a spindle receptor in time after application of a catelectrotonic current of $1 \times 10^{-7}$ A which flowed across a paraffin gap situated at 0.5 mm (▲), 3 mm (○), and 10 mm (●) from the capsule. The discharge frequency was given by the reciprocal of the interval of spikes, and plotted at the time of each spike after application of the current.

against time after the application of the current. When the current was applied to an axonal portion less than 1 mm away from the capsule, the discharge frequency increased from a normal level of 2.1 impulses/sec to 12 impulses/sec and it was maintained during the current application. When the current was applied at a portion 3 mm from the capsule, the frequency attained a peak of 70 impulses/sec at 350 msec and then decreased with a half decay time of 650 msec. A peak frequency of 120 impulses/sec and more rapid adaptation with a half decay time of 200 msec were observed following application of the current to an axonal portion 10 mm distant from the capsule. These results indicate that the application of amount of depolarizing current to the axonal portion distant from the capsule induced a higher frequency of discharge than when applied in the vicinity of the capsule, but the rate of adaptation of the discharges becomes faster with an increase in the distance between the capsule and the paraffin gap.

In a preparation in which the parent axon was crushed in the vicinity of the capsule, with a paraffin gap of 2 mm length situated 0.5 mm from the
spindle capsule, neither spontaneous discharges nor responses to stretch were observed. In such a case, polarizing currents applied across the gap should excite only the parent axon. Only a single spike or a few spikes were observed following application of 10 times the threshold current \((3.3 \times 10^{-8} \text{ A})\), regardless of the distance between the paraffin gap and the capsule. This implies that a train of spike discharges in a frog muscle spindle may be elicited by depolarization at the axon membrane in the capsule.

**FIG. 3** represents the effects of polarizing currents on spontaneous discharges of a spindle receptor. The muscle was lengthened to the +2 mm length to show the results distinctly. In this case the discharge frequency without polarizing current was 5.5 impulses/sec. The frequency was expressed by the average number of spikes during the 0.5 sec after the application of the polarizing current. When the anelectrotonic current was applied across the paraffin gap situated on the axon at 0.5 mm from the spindle capsule, the frequency of the spontaneous discharge was decreased almost linearly with an increase in the current intensity over \(1 \times 10^{-8} \text{ A}\) and the all discharges disappeared when the current was \(3.5 \times 10^{-8} \text{ A}\). The frequency was increased

![Graph](image-url)
with increases in the catelectrotonic current over $1.5 \times 10^{-8} \text{A}$, and attained a plateau with the current of $5 \times 10^{-8} \text{A}$. The frequency at the plateau was usually two to three times higher than that of the control. A similar course of frequency change during polarization was also observed when the gap-capsule distance was 1 mm, although the effects of polarization on the frequency were smaller than those at the distance of 0.5 mm. When the distance was 2 mm or more, the frequency was decreased linearly with increases of the anelectrotonic current and no discharge appeared with the currents over $5 \times 10^{-8} \text{A}$, while with catelectrotonic currents the frequency increased quite slowly below $3 \times 10^{-8} \text{A}$ but steeply over it.

**Effects of polarizing currents on discharge frequency at different initial lengths**

Fig. 4 shows effects of polarizing currents on the discharge frequency in a spindle receptor, when the muscle was lengthened in four steps from the in situ length (+0 mm) to +2, +4, and +6 mm, while the distance between the paraffin gap and the spindle capsule remained unchanged at 1.0 mm. The receptor discharged spontaneously at 0.8 impulses/sec at +0 mm muscle length.

![Graph showing frequency of discharges from a spindle receptor at different initial lengths](image)

**Fig. 4.** Frequency of discharges (ordinate) from a spindle receptor at different initial lengths of the muscle (+0 mm, +2 mm, +4 mm and +6 mm), during various amounts of catelectrotonic and anelectrotonic currents (abscissa).
without polarizing currents. The discharge disappeared with an anelectrotonic current of $2 \times 10^{-8}$ A while the frequency was increased with catelectrotonic currents of $3 \times 10^{-8}$ A or more. When the muscle was lengthened to $+2$ mm, the receptor discharged at 6.5 impulses/sec without polarizing currents. The discharge frequency decreased with anelectrotonic currents of more than $2 \times 10^{-8}$ A and the discharge disappeared with a current of $6 \times 10^{-8}$ A, while the frequency increased with catelectrotonic currents of more than $2 \times 10^{-8}$ A. The overall trend of the frequency response due to change of the polarizing currents seemed almost similar to that observed at $+0$ mm length of the muscle. The receptor discharged at 12 and 20.5 impulses/sec at $+4$ and $+6$ mm muscle length respectively without polarizing currents. The amount of decrease or increase in discharge frequency by a given current intensity was the same as in the muscle of $+2$ mm length. This result implies that the applied current may exert an equivalent effect to physiological generator current at the site of origin of the long-lasting propagated impulses.

Responses to muscle stretch during polarizations

In this series of experiments, the muscle was stretched by 2 mm from an initial length of $+4$ mm, with the paraffin gap fixed at a distance of 0.5 mm from the spindle capsule. A receptor, shown in Fig. 5, discharged at a mean frequency of 11.5 impulses/sec at the initial length (see the left end of the upper traces in each record of Fig. 5). The frequency increased to 15 impulses/sec during a catelectrotonic current of $1 \times 10^{-7}$ A (the left record in Fig. 5A). When the muscle was stretched by 2 mm at 7 mm/sec during the catelectrotonus, the frequency attained a peak of 53 impulses/sec at the completion of the muscle stretch and then decayed to a steady level of 25 impulses/sec 0.5 sec after the completion (the right record in Fig. 5A). During application of a catelectrotonic current of $2.8 \times 10^{-7}$ A, the receptor responded with spikes of a low amplitude at 30 impulses/sec (the left record in Fig. 5B). The low amplitude spikes are not abortive spikes but a modified conductive spikes, because the amplitude of the conductive spikes diminishes continuously with application of a gradually increasing current. In Fig. 5B, the deflection of the steady potential caused by larger polarizing currents were smaller than that by smaller amounts of current in Fig. 5A, due to the bridge circuit adjustment. The discharge frequency was uniformly increased up to 35 impulses/sec and was maintained during the muscle stretch (the right record in Fig. 5B).

The discharge in this receptor ceased with anelectrotonic currents of more than $8 \times 10^{-8}$ A (the left records in Fig. 5C and D). When the muscle was stretched during the anelectrotonus of $1 \times 10^{-7}$ A and $2.6 \times 10^{-7}$ A, the peak frequencies were 35 impulses/sec and 39 impulses/sec respectively at the completion of the stretch, and the steady frequencies were 20 impulses/sec.
FIG. 5. Spontaneous discharges during application of electrotonic currents (left hand records in each set) and responses to muscle stretch of 2 mm at 7 mm/sec during the electrotonus of the same amount as that in the left hand records (right hand records) recorded from a spindle receptor at the initial length of the muscle. A and B; during catelectrotonus of $1 \times 10^{-7} \text{A}$ and $2.8 \times 10^{-7} \text{A}$. C and D; during anelectrotonus of $1.3 \times 10^{-7} \text{A}$ and $2.8 \times 10^{-7} \text{A}$. Upper traces; discharges and potential deflection by electrotonus in which smaller deflections in B and D as compared to those in A and C were noted to be due to the balance in the bridge. Lower traces; amounts of electrotonic currents in left hand records, and tension developments during stretch of the muscle in right hand records. Calibration; 0.2 sec, 2 mV for upper traces, $1 \times 10^{-7} \text{A}$ for currents and 0.3 g for tension in lower traces.

and 8 impulses/sec respectively 0.5 sec after completion of the stretch (the right records in FIG. 5C and D).

FIG. 6 represents the effect of muscle stretch of 2 mm during polarizations on the discharge frequency of a spindle receptor. The receptor discharged at 12.5 impulses/sec at the muscle length of +4 mm without the polarizing current. The discharge frequency was increased to 36.5 impulses/sec with a catelectrotonic current of $3 \times 10^{-7} \text{A}$, while the discharge was abolished with anelectrotonic currents of more than $1.3 \times 10^{-7} \text{A}$, as illustrated with a solid line in FIG. 6. The peak and the steady frequencies during muscle stretch were 49 impulses/sec and 25 impulses/sec respectively, in the absence of polarization. The peak frequency was distributed between 42 and 66 impulses/sec under either the an- or catelectrotonus (filled circles in FIG. 6), while the steady frequency was increased under catelectrotonus and decreased under anelectrotonus (hollow circles and a dotted line in FIG. 6). If the steady frequency is termed 'static component' and the difference in frequency between the peak value and the static component is termed 'dynamic component' in accordance with JANSEN and MATTHEWS10, the dynamic component
SPINDLE DISCHARGES DURING POLARIZATION

FIG. 6. The frequency (filled dots) and the static component (hollow circles and dotted line) in responses to muscle stretch of 2 mm, and resting frequency (filled circles and solid line) of discharges in a spindle receptor at +4 mm initial length of the muscle, during application of various amounts of catelectrotonic and anelectrotonic currents.

becomes smaller under catelectrotonus but larger under anelectrotonus. It can be said that adaptation is slower under catelectrotonus but faster under anelectrotonus.

Effects of polarizing currents on abortive spikes

Abortive spikes of spindle receptors were observed after elimination of propagated spikes by crushing the parent axon at a point less than 0.5 mm away from the spindle capsule, in analogy with similar observations made previously. Before crushing the axon, propagated spike discharges from a spindle receptor appeared spontaneously at 2.4 impulses/sec at the in situ length of the muscle as shown in FIG. 7. Currents of $2 \times 10^{-8}$ A produced an increase in the discharge frequency of propagated spikes when catelectrotonic, and a decrease when anelectrotonic. The discharge disappeared with an anelectrotonic current of $5 \times 10^{-8}$ A (hollow circles in Fig. 7). After crushing the axon, abortive spikes occurred at a frequency of 4.5 impulses/sec. The frequency of the abortive spikes remained unchanged with currents of less than $2 \times 10^{-7}$ A (the center record in Fig. 7). Catelectrotonic currents of $2.3 \times 10^{-7}$ A
or more increased the frequency of the abortive spike (8.3 impulses/sec with $3 \times 10^{-7}$ A) (the right record in Fig. 7). The frequency was decreased with anelectrotonic currents of $2 \times 10^{-7}$ A or more and the abortive spike was abolished with $2.6 \times 10^{-7}$ A (the left record in Fig. 7). The above results may be due to a leakage of the polarizing current from the crushing point along the axon. In order to exclude this possibility, polarizing currents were applied to the intact preparations from which many abortive spikes were recordable. Intensification of catelectrotonus from $2 \times 10^{-8}$ A to $1 \times 10^{-7}$ A increased the frequency of conducted spikes which obscured abortive spikes. The conducted spikes were depressed under anelectrotonus of 1 to $2 \times 10^{-7}$ A, while the abortive spikes remained intact. The abortive spikes were abolished with anelectrotonic current of $3 \times 10^{-7}$ A or more. These results exclude the possibility mentioned above. It is concluded that the necessary current to produce a significant change in the frequency of abortive spikes was approximately ten times larger than that necessary to produce a change in frequency of the propagated spikes.
DISCUSSION

The present results show that a limited portion of the spindle nerve terminal possesses an ability to produce long-lasting discharges, but the parent axon more than 0.5 mm from the capsule does not. This is in good agreement with the recent report of Nakajima and Onodera\(^{15}\) that long-lasting repetitive discharges by constant current stimulation can be elicited at the axonal region near the soma of the slowly adapting stretch receptor in crayfish. The suggestion that the whole length of the parent axon, including the dorsal root, lacks the ability to produce long-lasting discharges is in line with an earlier report\(^{5}\) that no sustained antidromic discharges into a spindle can be provoked by a constant current stimulation of the dorsal root filament. It has also been demonstrated\(^{6}\) that conducted spike discharges in a spindle receptor are usually initiated at the first or the second node proximal to the ramification node but are sometimes initiated at more proximal nodes when the receptor is stimulated intensely. Such discharges adapt rapidly, because the axon does not have an ability to produce long-lasting discharges. This suggests that the absence of the ability to generate long-lasting discharges in the axon may be a mechanism for limiting excessive information transmission.

Grundfest\(^{3}\) has postulated that the mechanoreceptor consists of the accessory structure, the transducer membrane (electrically inexcitable) and the encoding membrane (electrically excitable). In the frog muscle spindle, intrafusal muscle fibers are considered to be the accessory structure, and the varicose threads in the form of chains of 'micro-spindles' the transducer membrane\(^{13}\). Since orthodromic and antidromic impulses have been known to conduct along the nonmyelinated terminals in Pacinian corpuscles\(^{4,16}\), the nonmyelinated axon membrane other than the varicose threads in the 'micro-spindles', and the peripheral nodes, may be responsible for the generation of impulses. The long-lasting discharges may not be attributed to a property in the accessory structure, because the discharge can be observed in a completely relaxed state of the denervated intrafusal muscle fibers\(^{10}\). The long-lasting discharge may not always follow a sustained depolarization (receptor potential) at the transducer membrane during stretch of the muscle, because such a depolarization has also been observed in a leaf-like receptor which responded with rapidly adapting discharges to muscle stretch\(^{6}\). This is in line with the report of Nakajima\(^{10}\) that there is essentially no difference in the time course of the generator potential between the rapidly and slowly adapting stretch receptor neurons in crayfish. In accordance with his view, it is concluded that the long-lasting discharge should be attributed to a property of the axonal membrane rather than to that in the terminal.

It is possible that long-lasting discharges are produced because the rate
of adaptation of the membrane at the terminal ramifying region is slow in comparison with that at the more proximal region along the stem axon. This possibility may be supported by the following facts: i) Adaptation, indicated by the amount of dynamic component of discharges during muscle stretch, is slow under catelectrotonus but fast under anelectrotonus. Since the axon membrane at the ramifying node or at the more distal part may always be depolarized by the generator potential, the adaptation of the membrane at these region may tend to be slower. ii) The present results suggest that the generator potential and the catelectrotonic current could sum effectively at the site of initiation of the conductive spike. If the long-lasting discharge is generated at a region other than the axon membrane, such a summation would not be expected. iii) When catelectrotonic currents over $1 \times 10^{-7}$ A were applied to a portion of the axon within a distance of 1.5 mm from the spindle capsule, a frequency plateau in the spindle discharge was observed; whereas when the currents were applied to the axon region 2 mm or more from the capsule, the frequency increased steeply. Since the length constant in the terminal axon of the frog spindle is about 2 mm\(^7\), the catelectrotonic current may greatly affect the site for generating long-lasting discharges in the gap-capsule distance within 1.5 mm, while acting mainly upon the site responsible for the resultant rapidly adapting discharges in the distance of 2 mm or more. iv) The current intensity which affect the discharge frequency of abortive spikes is approximately ten times greater than that for affecting the frequency of conducted spikes.

Another possibility is that the membrane property may be homogenous throughout the axon, but the surface of the terminal ramifying region in the sensory nerve may be closely surrounded by a structure, for example, by terminal Schwann cells, through which the long-lasting discharges may be transmitted to the axon. However, it would then be difficult to explain the fact that the discharge frequency of abortive spikes are modified by polarizing currents about ten times larger than the amount necessary to modify the frequency of propagated impulses.

**SUMMARY**

1. The effects of electrotonic currents on afferent nerve fibers isolated from the frog sartorius muscles were examined.
2. The spontaneous discharges were facilitated by catelectrotonus, and depressed by anelectrotonus. The frequency and adaptation of the facilitated discharges during catelectrotonus were lower and slower when a particular current was applied in the vicinity of the capsule than when applied to an axonal portion distant from the capsule.
3. Current-frequency relation curves obtained with different resting muscle
lengths were always in parallel with those obtained in situ length, with
certain difference of the frequency caused by the change in the muscle
length.
4. The static component in discharges during stretch of the muscle at 7 mm/
sec from an initial length was also increased with catelectrotonic and decreased
with anelectrotonic currents respectively, but the peak frequency in discharge
at completion of the muscle stretch appeared independent of the amount of
electrotonic currents applied. Consequently the dynamic component was de-
creased under catelectrotonus and increased under anelectrotonus.
5. After the axon was crushed at a portion close to the capsule, all the
spontaneous conducting discharges were abolished, and a single or few con-
ducting spikes could be elicited by the electrotonic currents.
6. The discharge of abortive spikes was increased or decreased by catelectro-
tonic or anelectrotonic currents of about ten times greater intensity than that
for affecting the propagated spike.
7. The above results suggest that the axonal portion distal to the ramifying
node possesses an ability for producing long-lasting discharges, and that the
ability is due to a slow adaptation of the sensory terminal membrane which
differs from that of the stem axon.

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