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RESPONSES OF ISOLATED GOLGI TENDON ORGANS OF THE CAT TO MUSCLE CONTRACTION AND ELECTRICAL STIMULATION

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SUMMARY

1. Responses of Golgi tendon organs isolated from cat tail muscles to contraction of muscle fibres inserting directly into the receptor (GTO-muscle fibres) as well as to pulses of electrical current applied extracellularly through the sensory axon were studied.

2. Analysis of the responses to GTO-muscle fibre contraction indicated that the active force developed by each muscle fibre constituted an equally potent input to the receptor in proportion to the developed force.

3. The sensitivity of a Golgi tendon organ remained almost constant with changes in muscle length up to a length where maximum active tension was developed (l_0). Beyond l_0, the sensitivity tended to decrease.

4. The absolute force threshold (passive + active) at l_0 for initiating an impulse in the afferent nerve was estimated for five preparations to be 4·5–14 mg. It was also demonstrated that the contraction of a single GTO-muscle fibre may initiate impulse discharge from the receptor.

5. A constant depolarizing current applied extracellularly to a GTO through its axon initiated a train of impulses, probably originating from a site near or within the receptor capsule. Analysis of responses to constant currents of various intensities suggested that a single impulse initiation site was involved.

6. During combined stimulation, responses of a tendon organ to GTO-muscle fibre contraction simply added to the response initiated by a constant current pulse, suggesting that the impulse initiation sites activated by each mode of stimulation were identical, or situated very close to each other in the nerve terminal.

INTRODUCTION

The Golgi tendon organ or GTO, one of the major receptors involved in the central control of muscle contraction, is a tension receptor that responds to contractions of certain number of motor units whose muscle fibres lie directly in series with the receptor (Houk & Henneman, 1967; Houk & Simon, 1967; Reinking, Stephen & Stuart, 1975; Jami & Petit, 1976). Based on the analysis of GTO responses to contraction of these motor units, the threshold force and sensitivity of GTOs have been calculated (Houk, Singer & Henneman, 1971; Binder, Kroin, Moor & Stuart,

1977). Based on the number of motor units acting on a single GTO in cat soleus and the number of muscle fibres in series with each receptor, Houk & Henneman (1967) have suggested that a motor unit contributes only one or two muscle fibres that actually insert into a tendon organ (also Reinking *et al.* 1975; Jami & Petit, 1976). More recently, Gregory & Proske (1979) examined the responses of GTOs to combined stimulation of different motor units acting on single receptors and demonstrated that the relation between firing rate and tension for combined stimulation may differ markedly, depending on the combination of motor units stimulated. Based on this and other findings they suggested that the response seen in the parent axon reflects the interaction of impulses originating from multiple sites along the sensory ending. Since the above studies on GTOs have used whole muscle preparations *in situ*, these approaches are obviously indirect. A more direct approach is to employ isolated preparations where various experimental parameters may easily be controlled.

Single tendon organs with or without muscle fibres inserting into them can be isolated from cat tail muscles, and will survive well *in vitro* for many hours. Information obtained from isolated single GTOs without muscle fibres attached has already been published (Fukami & Wilkinson, 1977). This report deals with analysis of responses of isolated single GTOs to GTO-muscle fibre contraction as well as to constant current pulses applied extracellularly through the sensory axon. Interaction between the responses to these two modes of stimulation was also examined. The results provide information on threshold force for initiating an impulse from the receptor, the sensitivity as a function of muscle length, and the site of impulse initiation in the sensory terminal. Evidence is also presented that single GTO-muscle fibre contractions can initiate impulse discharge from the receptor.

METHOD

Preparation

Cats were anaesthetized by I.P. injection of pentobarbitone (30 mg/kg). Immediately following transection of the skinned tail from the rest of the body, the dorsolateral tendon was retracted laterally to expose muscles to the bathing solution (composition in mM: NaCl 145, KCl 1·5, $CaCl_2$ 1·8, $MgSo_4$ 1·0, KH_2PO_4 1·0, HEPES buffer 5·0, glucose 5·0, pH adjusted to 7·4 at room temperature). Under a dissecting microscope single tendon organs at the musculotendinous junction can be identified. A part of the muscle containing a Golgi tendon organ and its nerve was dissected out together with a piece of the tail bone from which the muscle originates; the preparation was transferred to another small chamber where further dissection and experiments were performed. Using fine needles and scissors, a single GTO together with its nerve was first isolated from the surrounding connective tissue and tendon. Isolation of muscle fibres inserting directly to the receptor (GTO-muscle fibres) then proceeded from the tendon side toward their origin. The bathing solution was changed several times during the course of this dissection. After isolation, the piece of tail bone was firmly fixed by magnet-based pins to the bottom of the chamber, the end of the tendon tied to a strain gauge (sensitivity 0·5 μV/mg, compliance 20 μm/g, resonant frequency 800 Hz, drift $\pm$ 1·5 μV/hr) mounted on a micromanipulator, and the nerve lifted into oil for recording impulse activity using a pair of Pt wire electrodes. Another pair of Pt wire electrodes was placed on GTO-muscle fibres for stimulation.

Stimulation and recording

To stimulate GTO-muscle fibres, 1 msec electrical pulses of various intensities were applied directly to the muscle fibres through a pair of stimulating wire electrodes which, in order to reduce stimulus artifacts in nerve records, were placed away from the receptor, usually near the muscle origin. The technique employed for electrical stimulation of the sensory ending was similar to that

described by Edwards (1955) for frog muscle spindles and by Fukami (1970) for snake muscle spindles. A single sensory axon innervating a GTO was isolated and cleaned, and the receptor brought near the interface of the bathing solution and liquid paraffin. The length of the nerve extending between the GTO and an Ag-AgCl wire electrode was approximately 1 mm. Constant current pulses of various intensities and durations were passed from the electrode towards the receptor to depolarize the sensory terminal. The wire electrode was connected to an electrometer input (W-P Instruments Model M-707) which was designed for passing constant currents through a recording electrode by means of a bridge circuit. This electrometer was also used for stimulation of single GTO-muscle fibres by passing outward current pulses through an intracellular glass micropipette filled with 3M-KCl. Two Princeton Applied Research Model 113 amplifiers were used, one for nerve (both low and high frequency roll-off set at 1 kHz), and the other for tension. The outputs of the amplifiers, together with electrical current pulses applied through the nerve, were recorded on FM analog magnetic tape (Hewlett Packard 3960 Instrumentation Recorder). Intervals between successive impulses (interimpulse intervals) were measured on records reproduced from the tape.

Experiments were performed at room temperature of about 25 °C.

RESULTS

Responses to active force developed by GTO-muscle fibres

GTO-muscle fibres were stimulated near their origin with a 1 msec square pulse at 25–40 Hz for a duration of 1·5–2 sec. This was repeated once every 5 sec. The frequency of GTO impulse discharge during the plateau phase of a tetanic tension was measured on records reproduced from analog magnetic tape (Fig. 1*A*), and plotted as a function of plateau levels (Fig. 1*B*). The relation was generally similar to that obtained from isolated preparations without muscle fibres attached (Fukami & Wilkinson, 1977); Beyond a certain level of tension the slope of the relation tended to decrease. At any muscle length examined, however, the correlation coefficient by rank (r_s) between these two variables was in most cases larger than 0·9. The high value of r_s implies that the impulse frequency increases monotonically with the increase in tetanic tension, or with the number of GTO-muscle fibres activated. Thus individual GTO-muscle fibres may constitute almost equally potent inputs to the receptor, contributing in proportion to their developed force to the output (impulses) from the receptor. In Fig. 1*C* the coefficient of variation of the interimpulse intervals is plotted against the mean interval. The relation again appears to be monotonic ($r_s = 0{\cdot}90$, P [two-tail t test] $< 0{\cdot}001$). The sensitivity of a GTO at a given muscle length was estimated from the slope of a regression line for the initial portion of the tension *vs.* response relation (Fig. 1*B*), and related to the length–tension diagram obtained from the same preparation. An example is shown in Fig. 2 where the sensitivity (●) fluctuates more or less ($\pm 10{\cdot}5$ i.p.s./g) around the mean of 99·3 i.p.s./g up to 5 mm extension of the muscle. This fluctuation of sensitivity does not seem to be related to the muscle length ($r_s = 0{\cdot}28$, P [two-tail t test] $> 0{\cdot}1$), varying among preparations tested. The average sensitivity (mean $\pm$ S.D. in i.p.s./g) obtained from five preparations over muscle lengths from a just taut length to l_o, where maximum twitch tension developed, was $99{\cdot}3 \pm 10{\cdot}5$, $335 \pm 19{\cdot}1$, $281{\cdot}6 \pm 14{\cdot}2$, $169{\cdot}0 \pm 6{\cdot}3$ and $125{\cdot}0 \pm 8{\cdot}7$. This variation of sensitivity among preparations may reflect the variation of stiffness among individual GTOs: the stiffer a GTO, the less sensitivity is expected (Fukami & Wilkinson, 1977). The above values of sensitivity are three orders of magnitude higher than the value of 0·2 i.p.s./g obtained by

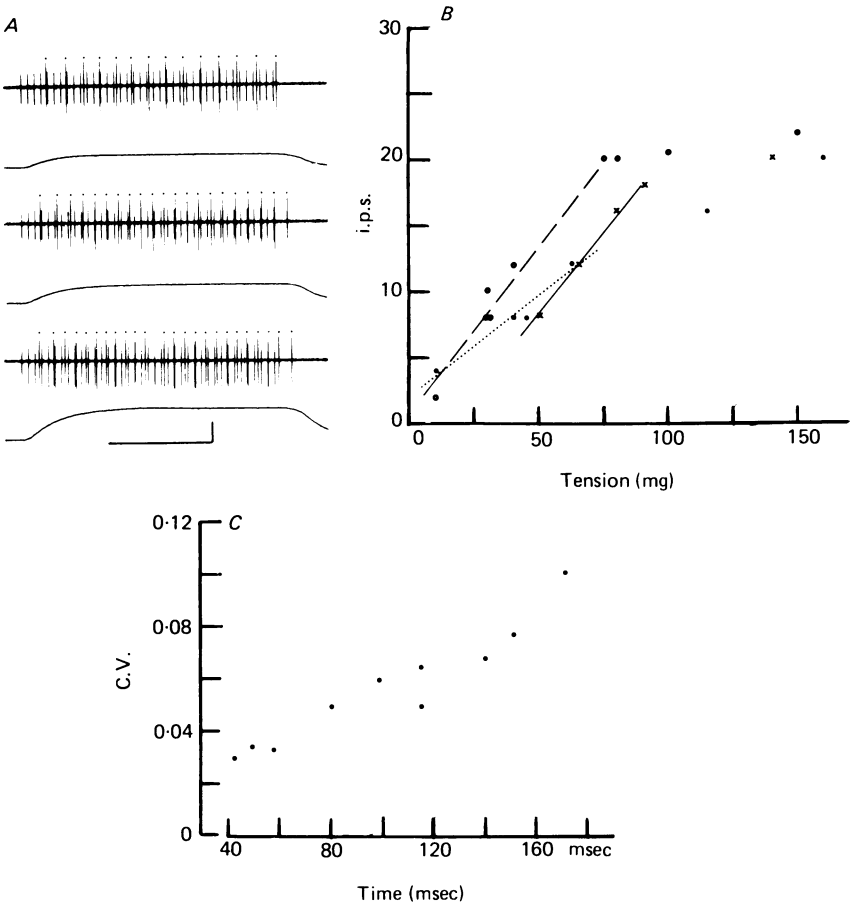

Fig. 1. Impulse discharge from an isolated GTO-muscle preparation in response to various levels of tetanic contraction of GTO-muscle fibres. *A*, three examples of single trace records where upper traces represent impulses and lower traces tension. Each impulse is marked by a dot. Other spikes are stimulus artifacts. The intensity of muscle stimulation at 32 Hz increased from top to bottom. Time calibration, 0·5 sec. Tension calibration, 100 mg. *B*, the relation of plateau tension *vs.* discharge rate. Crosses were obtained at a just taut muscle length ($\sim$10 mm, $\Delta l = 0$), open circles at 3 mm longer length ($\Delta l = 3$ mm), and filled circles at 5 mm longer muscle length ($\Delta l = 5$ mm) at which a sustained spontaneous discharge occurred. Correlation coefficient by rank (r_s), 1·0 for crosses, 0·975 (P [two-tail t test] $< 0{\cdot}001$) for open circles, and 0·886 ($P < 0{\cdot}001$) for filled circles. A regression line calculated for each group of data within 90 mg or less tension is also shown. The slope of these lines (sensitivity) is 240 i.p.s./g for the continuous line (correlation coefficient, $r = 0{\cdot}974$), 250 i.p.s./g for the dashed line ($r = 0{\cdot}987$), and 147 i.p.s./g for the dotted line ($r = 0{\cdot}982$). *C*, plot of coefficient of variation (C.V.) against mean intervals estimated by measuring 5–12 interimpulse intervals in single trace records at various plateau tension. $\Delta l = 3$ mm. $r_s = 0{\cdot}90$, $P < 0{\cdot}001$.

Gregory & Proske (1979) from the experiments in GTOs in medial gastrocnemius of cat by various combinations of tetanic stimulation of single motor units each of which, when stimulated singly, evoked a response from a GTO. This discrepancy is possibly due to the active force developed by muscle fibres of single motor units not in series but in parallel with the GTO, thus decreasing the sensitivity measured. Beyond l_0 the sensitivity tended to decrease. For example, the sensitivity of a GTO beyond l_0 decreased progressively from 281·6 to 160 i.p.s./g at 1·3 l_0 and 100 i.p.s./g at 1·5 l_0. This decrease may be ascribed to partial Na-channel inactivation at the impulse initiation site by prolonged depolarization of the sensory terminals and/or saturation of the receptor potential generating mechanism. Or, it may be mechanical in origin; if the compliance of a GTO decreases progressively beyond a certain level of tension, this would result in progressively less nerve responses to a given change in tension because of less extension of the nerve ending for a given increment of tension.

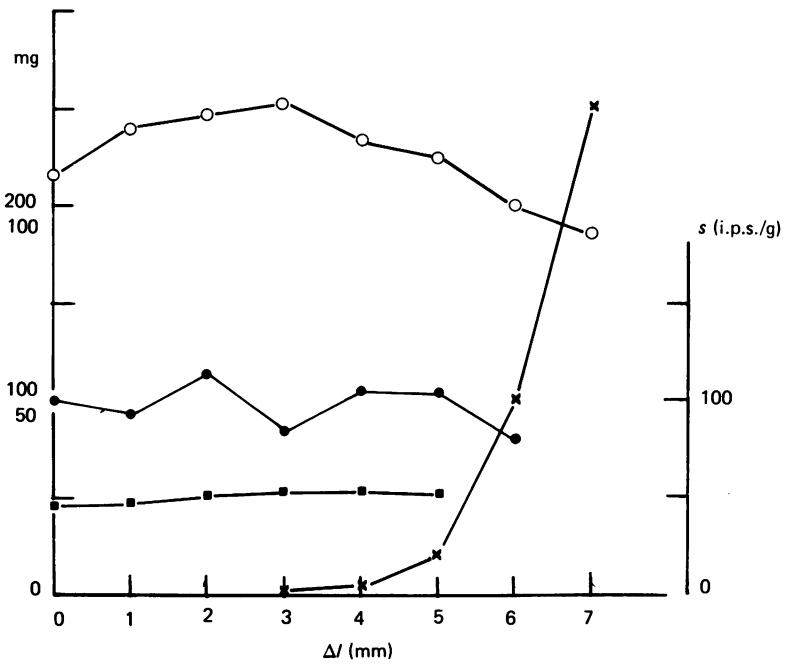

Fig. 2. Sensitivity and threshold active force for initiation of an impulse as a function of muscle length. ○, ×, maximum tetanic tension and passive tension, respectively (left ordinate, upper figures). ●, sensitivity, *s*, in i.p.s./g (right ordinate). ■, threshold active tension (left ordinate, lower figures). Abscissa, muscle length (Δl) relative to the just taut length (= 10 mm).

In another series of experiments the threshold active tension for initiation of an impulse was measured during the rising phase of a twitch contraction at the time when an impulse was initiated. In the GTO-muscle preparation shown in Fig. 2 the threshold (■) remained relatively constant over the muscle lengths examined. The absolute threshold (Binder *et al.* 1977) measured at l_0 for five preparations (active + passive tension in mg) was 1·5 + 3, 2·8 + 10, 11·3 + 3, 20 + 2 and 8·0 + 6.

In order to examine whether the active tension developed by a single GTO-muscle

fibre is capable of exciting a tendon organ, the muscle length was first adjusted to l_o. Using an intracellular glass micropipette in a bridge circuit, a single GTO-muscle fibre was stimulated. When stimulated intracellularly, muscle fibres with more than −60 mV resting potential responded with an all-or-none action potential accompanied by a clearly visible twitch contraction with a peak tension ranging from 20 mg to less than 2 mg (mean ± S.E.; 6·3 ± 6·1 mg, $n = 9$). Eight out of fifteen preparations thus examined showed impulse initiation from the receptor in response to twitch contraction of a single muscle fibre. The value for threshold active tension also varied greatly from preparation to preparation, ranging from less than 1–8 mg (mean ± S.E.; 2·6 ± 2·8 mg, $n = 9$). Some examples of records are illustrated in Fig. 3. It is quite remarkable that some GTOs *in vitro* are sensitive enough to be activated by a twitch contraction of a single GTO-muscle fibre. Considering the temperature coefficient (Q_{10} = 2·5–3·5, Fukami & Wilkinson, 1977) for static sensitivity of isolated GTOs *in vitro* as well as the larger twitch peak tension developed by a single muscle fibre *in vivo* (Binder *et al.* 1977), one may expect higher sensitivity and lower threshold tension for GTOs *in vivo*.

Responses to electrical stimulation

When the intensity of a constant current pulse passed through the axon from the recording wire electrode toward a tendon organ (depolarizing current) reached a certain level, a train of nerve impulses appeared. The site of initiation of these impulses was inferred to be situated either near or inside the capsule of the receptor from the following observations. First, when the axon was crushed near the capsule or the receptor mechanically destroyed, the discharge disappeared. Secondly, when the interface between bathing solution and oil was raised so that the peripheral portion of the axon was in the bathing solution or, alternatively, when the peripheral part of the axon was laid slack on the receptor capsule, a sharp increase in the threshold current intensity for initiating a train of discharge occurred. An example of responses to a constant depolarizing current lasting more than 2 min is shown in Fig. 4 where impulse discharge continued throughout the duration of the applied current. As plotted in *B* the mean rate of discharge measured over consecutive 1·5 sec periods slowly decreased before it reached a steady value of about 7 impulses/sec. Since a constant current was applied, it seems safe to assume that the recorded impulses were initiated from a single site in the terminal, and that slowly developing adaptation of the response occurred at that site. This might be due to extracellular potassium accumulation which may activate an electrogenic sodium pump or K-Na exchange pump (Van Essen, 1973), and/or 'ultra-slow' inactivation of Na currents described by Fox (1976). The average of ten consecutive interimpulse intervals increased with time during the adaptation period (Fig. 4*C*). Furthermore, a scatter plot of the coefficient of variation of ten consecutive intervals (measured for up to 60 sec) against mean intervals (Fig. 4*D*) showed a monotonic relation between these two variables ($r_s = 0{\cdot}64$, $P > 0{\cdot}01$) without any obvious abrupt discontinuity which might indicate involvement of different impulse initiation sites during the adaptation process (Brokensha & Westbury, 1974).

As expected, an increase in depolarizing current intensity increased the rate of discharge from the organ (Fig. 5*A*). The relation between rate of discharge and

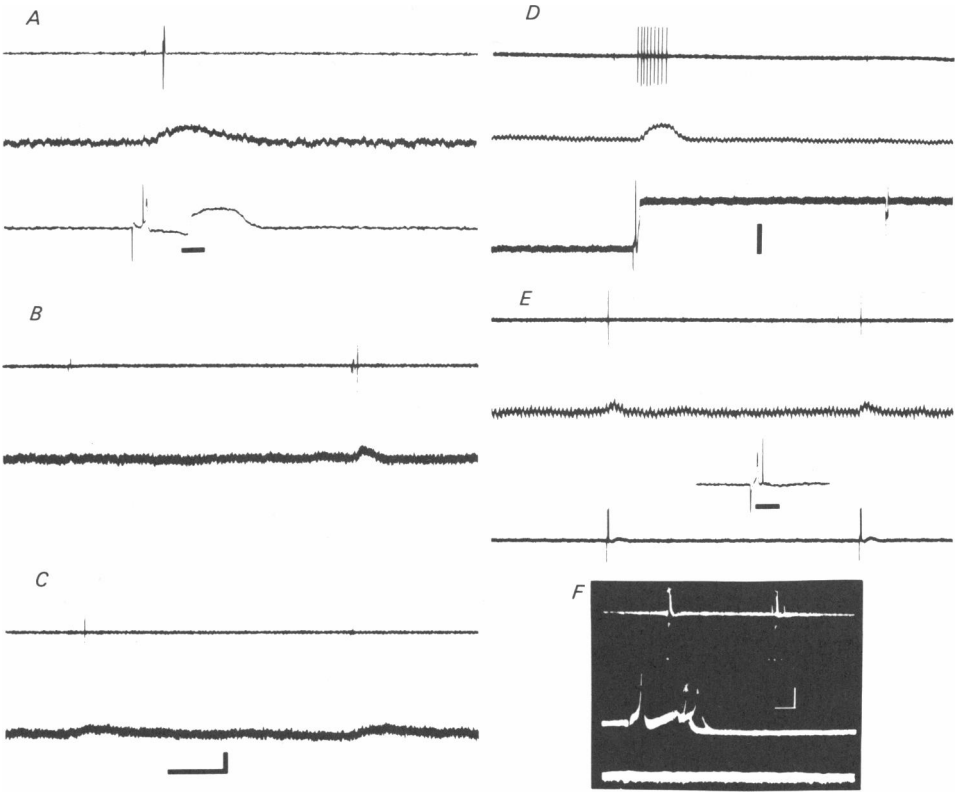

Fig. 3. Impulse discharge from a GTO in response to intracellular stimulation of a single GTO-muscle fibre. Records *A*, *B* and *C* represent respectively responses of a GTO-muscle preparation to intracellular stimulation of three different muscle fibres. In *A*, a muscle action potential shown in the bottom trace (stimulus artifacts seen as downward and upward deflexion preceding the action potential) caused a twitch contraction (middle trace) and an impulse discharge from the organ (top trace). In *B*, the first stimulation of a second fibre failed to initiate any response whereas the second stimulus produced a twitch (bottom trace) and an impulse (top trace). In *C*, the first twitch contraction initiated an impulse but the second one failed to do so. In *B* and *C* intracellular records were omitted. Resting potential $\simeq -65$ mV for all three fibres. Records *D* and *E* represent records taken from two out of three impaled fibres of another GTO-muscle preparation. In *D*, the intracellular electrode following a muscle action potential got out of the cell (bottom trace) causing muscle contracture (middle trace) and a train of impulse discharge from the organ. Note that the same stimulating current when applied extracellularly caused no response at all. Inset record in *E* shows the muscle action potential at a faster time base (time calibration, 10 msec). Resting potential $\simeq -80$ mV for both fibres. In record *F*, in response to suprathreshold stimulation of a muscle fibre, two muscle action potentials (middle trace) and two nerve impulses from the organ (top trace) were initiated for each trial without any noticeable tension development (bottom trace). Four single trace records were superimposed. Resting potential $\simeq -70$ mV. Time calibration in *C* (250 msec) applies to *B*, *C*, *D* and *E*. Tension calibration in *C*, 5 mg for *A*, *B*, *C* and *E* and 10 mg for *D*. Voltage calibration in *D* for intracellular recordings, 100 mV for *A* and *E* and 50 mV for *D*. Time calibration in *A* and *F*, 10 msec. Vertical bar in *F*, 50 mV for intracellular recording and 5 mg for tension.

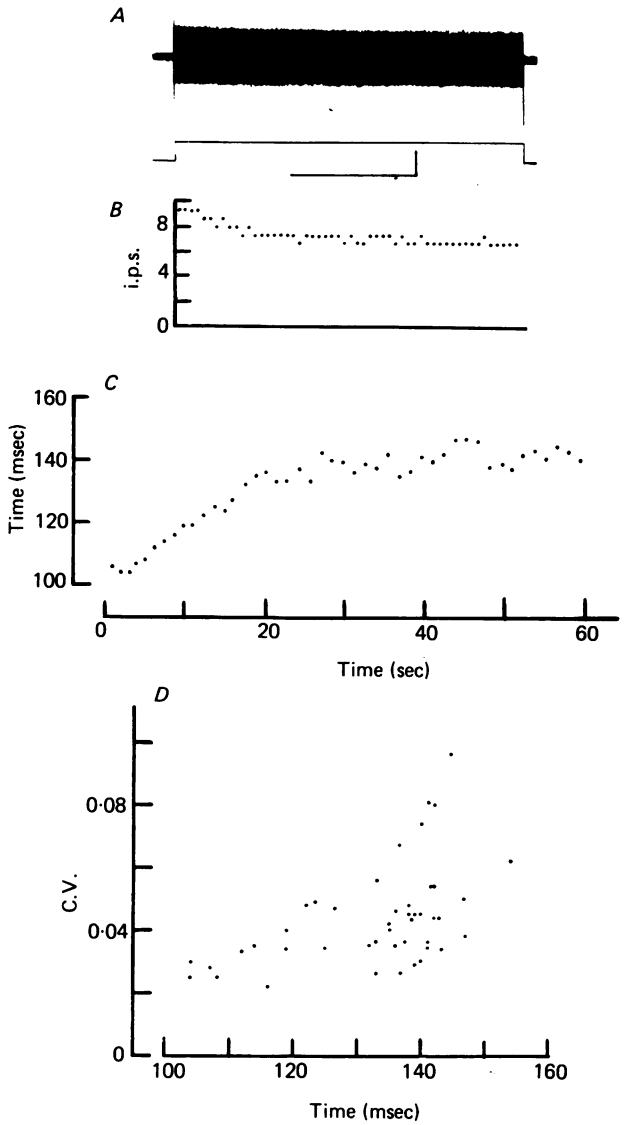

Fig. 4. Impulse discharge from an isolated Golgi tendon organ in response to a constant current applied through the innervating axon. As shown in the upper record in *A* impulse discharge continued throughout the entire period (~80 sec) of current application (lower trace in *A*). Time and current calibration in *A*, 30 sec and 10^{-7} A, respectively. In *B* the mean discharge rate determined by counting the number of impulses during consecutive 1·5 sec periods is plotted on the same time scale as in *A*. *C*, mean interimpulse intervals of ten consecutive impulse intervals measured up to 60 sec from the start of current application. *D*, scatter plot of coefficient of variation (C.V.) of ten consecutive interimpulse intervals against mean impulse intervals during the same period as in *C*. Correlation coefficient by rank, $r_s = 0{\cdot}64$, P (two-tail t test) $< 0{\cdot}01$.

current intensity is illustrated in Fig. 5*B* where each point was calculated from measurement of 70–217 interimpulse intervals included in five to ten single trace records obtained for a given current. As shown by the four examples of interimpulse interval histograms in Fig. 5*C*, the regularity of intervals gradually decreased with the decrease in current intensity, and the distribution gradually became skewed towards longer intervals with increasing mean intervals. Similar observations have

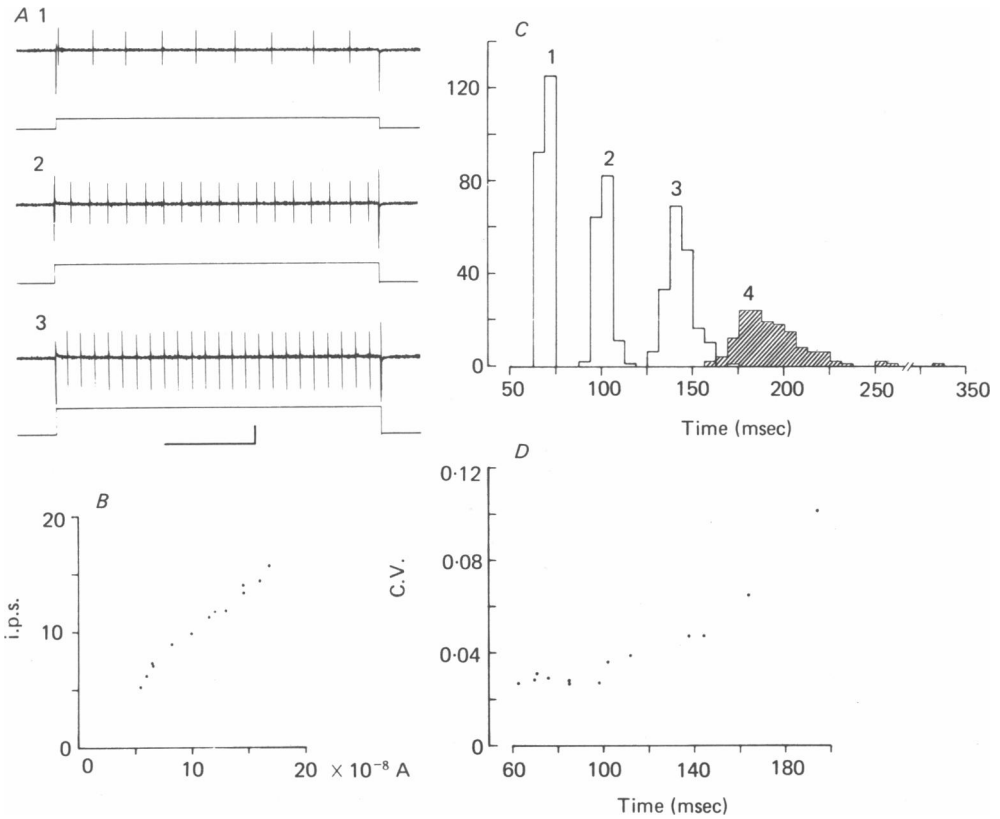

Fig. 5. Responses of an isolated tendon organ to electrical pulses of various intensities. Examples of single trace records are shown in *A* where the rate of impulse discharge (upper trace) is seen to increase from *A*1 to *A*3 with increasing current intensity (lower trace). The increase in impulse amplitude with the increase in current intensity (*A*1–*A*3) is apparently due to the applied current which depolarizes the axon terminal, but at the same time hyperpolarizes the axonal membrane near the recording electrode. Time and current calibration, 0·5 sec and 10^{-7} A, respectively. *B*, plot of mean rate of discharge (i.p.s.) against current intensity. Each data point was obtained by measuring 70–217 intervals contained in 5–10 single trace records for a given current intensity. $r_s = 0{\cdot}986$, *P* (two-tail *t* test) $< 0{\cdot}001$. *C*, examples of interimpulse interval distribution of responses to various current intensities. Bin width, 6·25 msec. Ordinate, number of intervals. Current intensity, mean interval ± S.D. and coefficient of variation (C.V.) for each histogram are; 16×10^{-8} A, 69·9 ± 2·0 msec ($n = 217$), C.V. = 0·029 for histogram 1, 10×10^{-8} A, 102·0 ± 3·7 msec ($n = 185$), C.V. = 0·036 for histogram 2, $6{\cdot}6 \times 10^{-8}$ A, 143·7 ± 6·8 ($n = 185$), C.V. = 0·047 for histogram 3 and $5{\cdot}5 \times 10^{-8}$ A, 193·9 ± 21·5 msec ($n = 145$), C.V. = 0·111 for histogram 4. *D*, coefficient of variation plotted as a function of mean interimpulse intervals. $r_s = 0{\cdot}810$, *P* (two-tail *t* test) $< 0{\cdot}01$.

been reported for many other sensory receptors including frog muscle spindles (Buller, Nicholls & Strom, 1953; Buller, 1965; Brokensha & Westbury, 1974), snake muscle spindles (Fukami, 1970) and optic nerve fibres of *Limulus* (Ratliff, Hartline & Lange, 1968). To assess the variability of interimpulse intervals, the coefficient of variation for each data point in Fig. 5*B* was plotted against the mean interval (Fig. 5*D*). There were no obvious abrupt changes in the relation between these two parameters, the coefficient of variation increasing smoothly with the increase in mean intervals ($r_s = 0{\cdot}81, P < 0{\cdot}01$). As discussed by Brokensha & Westbury (1974), if the relationship between the variability and the mean rate of discharge (or interimpulse intervals) were different for each impulse generator, then the lack of any abrupt change in this relation would indicate a single origin of the impulse train.

Interaction between responses to muscle contraction and to electrical stimulation

The above results raise the question of whether impulses elicited by active muscle force and those initiated by electrical pulses do originate from the same site or from different sites along the sensory ending. In the former situation where the same site is involved, depending upon the distance between the impulse initiation site and the sensory transduction site in the terminal, two possibilities may be considered. If the two sites were situated close to each other, then the responses during combined stimulation might be less than simple addition of responses to each mode of stimulation because of the increased conductance during the receptor potential which would short circuit the applied current. In contrast, if the two sites were situated far apart, then the response to combined stimulation would be a simple summation of responses to each mode of stimulation. In the latter situation where more than one source is involved, one may expect to see evidence for interaction of impulses originating from two independent sources such as resetting (Matthews, 1931; Fukami, 1980), increase in both mean frequency and regularity of discharge (Eagles & Purple, 1974; Fukami, 1980), and/or irregular discharge due to impulse collision along terminal branches or probabilistic mixing of impulses (Clifford & Sudbury, 1972; Fukami, 1980). The above possibilities were tested by independent analysis of the response to each mode of stimulation, and analysis of the response to combined stimulation. It was found that the discharge rate in response to GTO-muscle fibre contraction simply added to the response to electrical stimulation. Example records of this experiment are shown in Fig. 6 where the top record *A* shows the response to a constant current, record *B* the response to a tetanic contraction of GTO-muscle fibres, and the bottom record ($A+B$) the response during combined stimulation. During combined stimulation the rate of discharge increased gradually, reaching a rather regular firing rate which appears higher than that of the response shown either in *A* or *B*. This finding suggests the absence of both resetting and impulse mixing during this period. For further analysis, instantaneous frequency of discharge in response to each mode of stimulation as well as to combined stimulation were plotted as a function of time measured from the onset of current application. Two sets of examples are shown in Fig. 7 where each graph represents superimposed plots of measurements made on two to four single trace records of the response to electrical stimulation, the response to tetanic contraction of GTO-muscle fibres and the response to combined stimulation. As seen in this Figure, the response to tetanic

contraction during combined stimulation, was simply superimposed on top of the response to electrical stimulation. To examine this point further, the average rate of discharge was determined during the period of 800–1200 msec following the onset of current application when the response to tetanic contraction appears relatively stable. The value thus obtained for the response to combined stimulation was compared with the value for the response to tetanic contraction alone plus that for

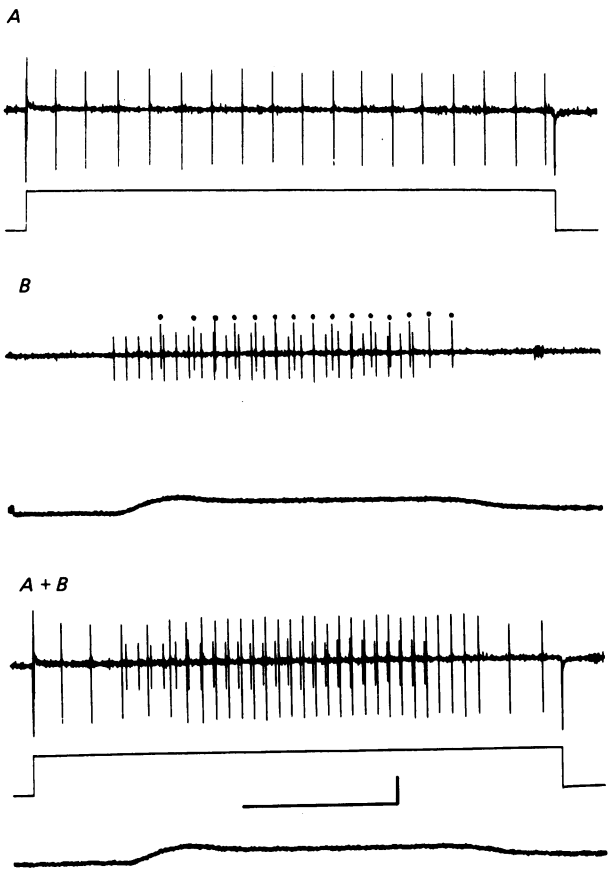

Fig. 6. Sample records illustrating the response to applied current (*A*), the response to tetanic stimulation at 25/sec of GTO-muscle fibres (*B*) and the response to combined stimulation (*A* + *B*) of an isolated GTO-muscle preparation. Upper trace in each record represents axonal discharge. Time calibration, 0·5 sec. Vertical calibration bar, 9×10^{-8} A for current and 50 mg for tension. Each impulse in *B* is marked by a dot. Other spikes in *B* and smaller spikes in the upper trace in *A* + *B* are stimulus artifacts.

the response to current stimulation. These two values for each set of graphs in Fig. 7 were not significantly different ($P > 0{\cdot}1$ for the left set, $P > 0{\cdot}05$ for the right set of graphs). Furthermore, the test for variance ratio (F test) examined in the similar way indicates that the impulse frequency distribution during combined stimulation is not significantly different from that of the response to each stimulation added together during the same period ($P > 0{\cdot}25$ for the left, $P > 0{\cdot}1$ for the right set of graphs). Taken together, these findings indicate that impulses elicited by muscle

contraction and by electrical stimulation originate from an identical site in the sensory terminal, or that the sites responding to each mode of stimulation are situated so close to each other that no impulse interaction occurs. Closer examination of Figs. 6 and 7 also reveals that the impulse activity during the initial portion of muscle contraction is seen during combined stimulation as an initial smooth rise of the rate of discharge that is absent in the response to muscle contraction alone. This suggests that the subthreshold excitatory process or receptor potential caused by muscle contraction summates at the impulse initiation site with the depolarization caused by an externally applied current.

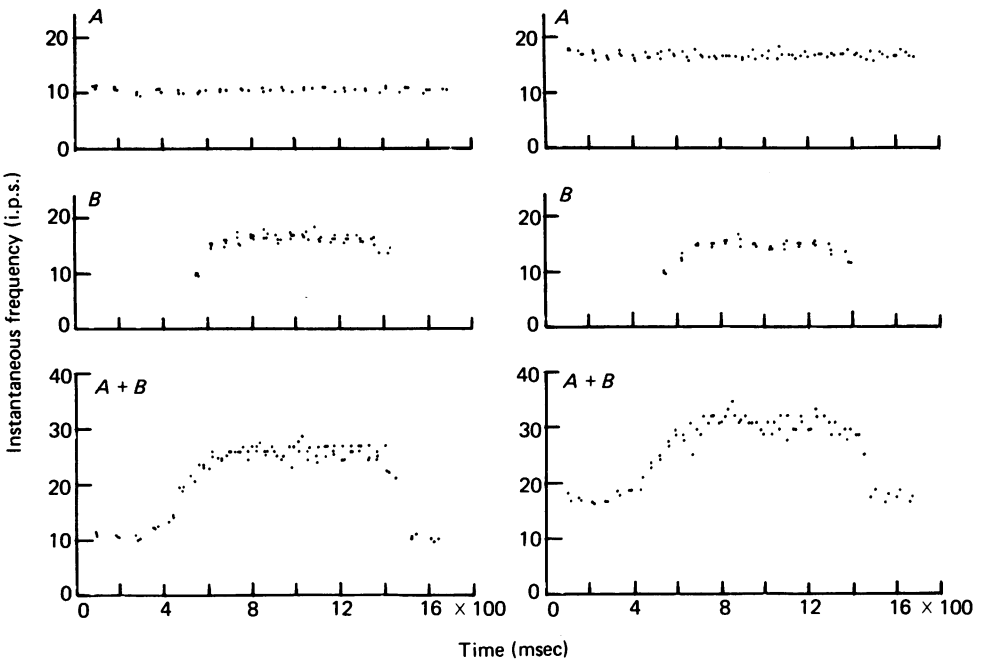

Fig. 7. Instantaneous rate of impulse discharge plotted against time measured from the onset of current application. *A*, the response to a constant current pulse. *B*, the response to tetanic contraction of GTO-muscle fibres. *A* + *B*, the response to combined stimulation. The number of single trace records superimposed, 3 for each upper plot, 4 for the middle left and 3 for the middle right, 3 for the bottom left and 2 for the bottom right. Single trace records corresponding to each plot in the left column are shown in Fig. 6. The mean impulse frequency (mean ± S.D.) during the period between 800 and 1200 msec for data in the left column, 10·6 ± 0·4 (n = 12) for *A*, 16·6 ± 0·8 (n = 20) for *B* and 25·8 ± 1·2 (n = 31) for *A* + *B*. Corresponding values for those in the right column, 16·7 ± 0·7 (n = 20) for *A*, 14·9 ± 0·6 (n = 18) for *B* and 30·9 ± 1·5 (n = 25) for *A* + *B*.

DISCUSSION

By assuming that about one third of the threshold tension to stretch (200 g in soleus muscle of the cat) is supported by muscle fascicles, and that 10 muscle fibres are inserted upon each tendon organ (Barker, 1967) while 24,000 muscle fibres are in soleus, Houk *et al.* (1971) calculated the threshold force for sustained afferent discharge to be as low as 28 mg. More recently, Binder *et al.* (1977), with similar

assumptions, estimated the absolute threshold force for initiation of a single impulse from a GTO in cat soleus muscle to be as little as 4 mg. The corresponding values obtained from isolated preparations *in vitro* are approximately 8–170 mg for a sustained discharge (Fukami & Wilkinson, 1977) and 4·5–22 mg for initiation of a single impulse. Although the properties of GTOs *in situ* may differ from those described in the present work, the two sets of values obtained from *in situ* and *in vitro* preparations are remarkably similar, both pointing toward high sensitivity of this sense organ.

In their recent studies on GTOs in hind limb muscles of cats, Gregory & Proske (1979) reported that the relation between firing rate and tension for combined stimulation of up to twelve motor units acting on single GTOs may differ markedly depending on the combination of motor units stimulated. Based on these and other findings they proposed that each motor unit acts on a particular collagen bundle in a GTO, activating the impulse initiation site in the myelinated terminal branch innervating that collagen bundle. In other words each collagen bundle in a tendon organ behaves relatively independently of the others and the response seen in the parent axon reflects the interaction of impulses originating from multiple sites in the ending. In contrast, the present results (Fig. 1) suggest that contraction of each GTO-muscle fibre constitutes an equally potent input to the receptor, depending only upon the amount of active force developed; a tendon organ responds simply to the total force exerted upon it. This discrepancy seems to be due largely, if not entirely, to the differences in experimental conditions. For instance, a part of the results by Gregory & Proske (1979) could be explained, at least qualitatively, by assuming an unloading effect exerted by non GTO-muscle fibre contraction of single motor units (Houk & Henneman, 1967; Stuart, Mosher, Gerlach & Reinking, 1972). If only one or two muscle fibres of a single GTO-activating motor unit inserted directly into the organ (Houk *et al.* 1971), the larger the motor unit the more powerful the expected unloading effect. Smaller motor units might exert little such effect. Thus, when the largest motor unit is stimulated first, which would presumably produce a relatively weak tendon organ response, the response to successive recruitment of smaller motor units in order of increasing potency would result in a progressively steeper tension vs. response relation. On the other hand, the reverse order of recruitment would produce a relation showing a steep rising foot followed by a progressively less steep curve (Fig. 3 of Gregory & Proske, 1979). Similarly, when the firing rate evoked by each of a number of motor units of similar size differs by a relatively small amount, an approximately linear relation is expected between firing rate and tension, irrespective of the order of motor unit recruitment.

The dynamic response of GTOs where myelinated terminal branches also occur (Barker, 1974) is generally much less than that of spindle primary endings, being comparable to that of the secondary ending. Based on the analysis of receptor potentials and impulse discharge from single GTOs *in vitro* it has been suggested that it is not necessary to postulate functionally distinct multiple impulse initiation sites to account for the dynamic response (Fukami & Wilkinson, 1977).

The present analysis of responses to tetanic contraction of GTO-muscle fibres, to electrical current pulses and to combined stimulation suggests that the impulse discharge seen in the parent axon originates from a single impulse initiation site in

the terminal. This conclusion, however, does not exclude the possibility of multiple impulse initiation sites in myelinated branches as shown for frog muscle spindles (Ito & Vernon, 1975); each branch, if tested individually, might initiate impulses. The impulse initiation site examined in the present study might simply be the lowest threshold site among others and under the present experimental conditions only this site was activated. Or, as in the case of cutaneous type I mechanoreceptors (Horch, Whitehorn & Burgess, 1974), the highest rate of discharge initiated from the lowest threshold site may have suppressed discharge in other sites by a resetting mechanism. It is also possible that all myelinated terminal branches in a tendon organ are not excitable, subserving only electrotonic transmission of information from receptor sites to the impulse initiation site where information carried by all branches would be integrated. The short internodal length (0·5 mm or less) usually seen along myelinated terminal branches in a tendon organ is obviously unfavourable to electrotonic transmission. This disadvantage might be overcome by frequent terminal arborization which would increase the receptive membrane area where mechanoelectric transduction occurs, leading to a proportionate increase in the sensitivity of this sense organ. In addition, as indicated by computer simulation studies by Revenko, Timin & Khodarov (1973), the impedance mismatch resulting from the increased area of non-myelinated terminal membrane may be reduced because of decreased internodal length proximal to the terminal and decreased diameter of the terminal non-myelinated segment, leading to greater efficacy of electrotonic transmission along terminal branches.

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