J. Physiol. (1986), **376**, *pp.* 281–297
With 8 text-figures
Printed in Great Britain

STUDIES OF CAPSULE AND CAPSULAR SPACE OF CAT MUSCLE SPINDLES

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(*Received 18 July 1985*)

SUMMARY

1. The presence of hyaluronate in the capsular space of the cat muscle spindle was demonstrated using alcian blue staining at various pHs, the critical electrolyte concentration technique and hyaluronidase treatment.

2. In spindles with intact capsules an extracellular marker, the dye Ruthenium Red, gained access to the capsular space through the gap in the sleeve region, but for a limited distance. In muscle spindles with the capsule nicked, the marker diffused into the capsular space in the equatorial region, revealing a dense network in this space which consisted of globular structures interconnected by thin filaments. Based on their thickness, these filaments were inferred to be hyaluronic acid, and the globular structures were inferred to be protein molecules. Longitudinal diffusion of the dye into the capsular space through the nicked site was limited. The limited diffusion is probably due to electrostatic binding of the dye, which is a hexavalent cation, to negatively charged glycosaminoglycan hyaluronate that is present in the space.

3. The transcapsular potential was measured by use of glass micropipettes filled with 3 M-KCl. The value was 15 mV $\pm$ 4 (average $\pm$ S.D., $n = 12$; range, 10–20 mV) inside negative. The input resistance and capacitance of the capsule, measured with two independent electrodes, varied widely (1·3–8·0 MΩ and 0·5–1·3 nF, $n = 4$) and the capsule showed marked delayed rectification to outward current pulses. $[K^+]$ in the space measured with K^+-sensitive resin-filled glass micropipettes was a few millimolar higher than that in the bathing solution.

4. The effects of $[K^+]$ and $[Ca^{2+}]$ on impulse activities were examined in spindles with intact capsules or with partially resected capsules. In spindles with intact capsules the effects of $[K^+]$ and $[Ca^{2+}]$ were significantly less or negligible compared with those in spindles with the capsule opened.

5. Hyaluronidase ($\sim 10^{-4}$ g/ml) added to the bathing solution around nicked capsules significantly reduced both resting and stretch-induced impulse activities in 40–50 min. By this time the capsular space was completely collapsed. An increase in $[K^+]$ of the bathing solution from 3·5 to 6 or 8 mM restored these impulse activities. A similar restoring effect was also observed when $[Ca^{2+}]$ in the bathing solution was reduced. Based on the above findings it is proposed that the negatively charged glycosaminoglycans or hyaluronate present in the capsular space are responsible, at least partially, for the transcapsular potential, and more significantly, for the

decreased potential difference across the sensory terminal membrane, thus making the sensory ending sensitive to mechanical stimuli.

6. It was concluded that the spindle capsule and hyaluronate present in the capsular space play an important role in regulating the ionic milieu around the sensory endings and maintaining their sensitivity to mechanical stimuli.

INTRODUCTION

In spite of numerous studies of muscle spindles, relatively little attention has been paid to the capsule and the capsular space of muscle spindles. Sherrington (1897), on the basis of dye injection experiments, maintained that the capsular space is continuous with the lymphatic space, being filled with lymph. The nature of this fluid-filled space and the functional significance of the capsule are controversial. Several investigators (Jahn, 1959; Voss, 1962) favour Sherrington's notion of the lymph-filled space. Brzezinski (1961*a*, *b*), by histochemical studies, demonstrated that the space is filled with a glycosaminoglycan, hyaluronic acid. He suggested that these viscous complexes protect the sensitive nerve endings against non-specific stimulation, for instance, from movements of the perimysium and the whole muscle. Conversely, Bridgeman & Eldred (1964) proposed that the mammalian spindle and its thick capsule may function, in part, as a pressure-sensitive device and that pressure changes on the capsule could be transmitted to the sensory endings by stretches of the intrafusal fibres. Poppele, Kennedy & Quick (1979) compared the mechanical properties of intrafusal fibres in cat spindles before and after removal of the capsule, and found no significant differences. Similar results were also obtained by Hunt & Wilkinson (1980). Shantha, Golarz & Bourne (1968), on the basis of histological and histochemical studies on the capsule of muscle spindles of guinea-pig and cat, suggested that the spindle capsule cells and perineural cells are both ectodermal in origin, sharing a common enzymatic profile (dephosphorylating and oxidative enzymes). Kennedy & Yoon (1979) tested the permeabilities of capillaries and capsules of rabbit muscle spindle to horseradish peroxidase (HRP). Intra-arterial HRP failed to cross spindle capillaries, as with capillaries in peripheral nerve. Kennedy & Yoon also showed that the inner layers of perineural cells of the spindle capsule are a barrier to HRP applied to the outside. However, tracers did enter between the outer two or three layers, in a way similar to that observed for nerve perineurium by Olsson & Reese (1971). More recently, Fukami (1982) demonstrated the presence of glycosaminoglycans in the capsular space of snake spindles, together with the finding of a transcapsular potential of $\sim$ 10 mV negative inside the space and some electrical properties of the capsule. The capsule cells were also shown to act as a diffusion barrier to HRP and an extracellular marker, Ruthenium Red.

The purpose of the present experiments was to elucidate some morphological and functional aspects of the capsule and capsular space of cat muscle spindles, using electron microscopy and histochemical as well as electrophysiological techniques.

METHODS

General

Muscle spindles in adult cat tail muscles were used. Dissection of the muscle and isolation of spindles under a dissecting microscope have been described elsewhere (Fukami, 1984). A single isolated spindle together with its innervating nerve was placed in an experimental chamber containing a modified Locke solution (in mM: NaCl, 145; KCl, 3·5; $CaCl_2$, 1·8; $MgSO_4$, 1·0; HEPES, 5·0; pH adjusted to 7·4 at room temperature). To expose the capsular space to the bathing solution (when desired) the capsule was nicked or partially resected using 26 gauge needles. The experiments were performed at room temperature (25 °C ± 2).

Morphological

For histochemical demonstration of glylcosaminoglycans in the capsular space single spindles together with some extrafusal fibres were soaked in 2·5 % DMSO (dimethyl sulphoxide), which was used as a cryoprotective agent Dalgliesh, Mellors & Blight, 1980; Gregory, 1980), in Locke solution for 15–30 min, and then sandwiched between pieces of cat liver for support. Frozen sections of 10 μm thickness were cut, mounted on glass slides, fixed in Carnoy fixative for 10 min, rinsed in 95 % ethanol, and air dried. The preparation was then subjected to alcian blue staining at varying pH and counter-stained by the periodic acid Schiff reaction. Prior to alcian blue staining some spindles were treated with varying concentrations of $MgCl_2$ (the critical electrolyte concentration technique, Scott & Dorling, 1965) or with hyaluronidase of 100–200 μg/ml (Sigma Type VI).

For electron microscopic studies an isolated spindle was fixed in a bath with 2·5 % glutaraldehyde in 0·1 M-cacodylate buffer, pH 7·4 for 1 h. The fixed spindle was soaked for 1–2 h in OsO_4–Ruthenium Red solution (Luft, 1966). The preparation was then subject to graded dehydration and embedding in Epon 812. Thin sections were examined without further staining in a Zeiss EM 10A electron microscope.

Physiological

For experiments with glass micro-electrodes a single spindle together with some extrafusal fibres on both sides was dissected and placed in a chamber containing modified Locke solution which was grounded via an Ag–AgCl wire or Ag–AgCl agar bridge. The extrafusal fibres acted as a support preventing rotation of the spindle capsule around its long axis during penetration. For the study of electrical properties of the capsule two independent micropipettes filled with 3 M-KCl (resistance, 60–70 MΩ), one for passing current and the other for recording potential, were introduced into the capsular space.

To measure $[K^+]$ in the space, one electrode filled with 3 M-KCl and a second filled with K^+-sensitive resin were inserted into the space. The K^+-sensitive electrode was fabricated according to the procedure of Reuss & Weinman (1979). The outputs from these two electrodes were fed to high impedance (10^{15} Ω) probes and the potentials were recorded on a strip chart recorder. The micro-electrodes, when filled with 3 M-KCl, had tip potentials of less than 10 mV. In other experiments an isolated spindle was placed in a chamber with one end tied to a semiconductor transducer (AE 802, Akers Electronics, Horton, Norway) for measurement of tension. The sensitivity of the transducer assembly was 40 μV/mg and its resonant frequency about 300 Hz. The transducer was excited by a d.c. bridge and the output was amplified through a d.c. amplifier. The other end of the spindle was tied to a rod attached to a servo pen recorder (Brush Instruments, Brush, Gould Inc., Cleveland, OH, U.S.A.) for recording of stretch. Signals from the motor's internal feed-back transducers provided a voltage proportional to the displacement of a stretching rod (Wilkinson & Fukami, 1983). Stretch was given once every 14 s. The nerve was lifted into oil on two platinum wire electrodes to monitor spindle sensory discharge. Sensory discharges were analysed in terms of background, static and dynamic peak frequency. The background discharge rate was determined by counting the number of impulses just before stretch, the static discharge rate by counting the number of impulses during the last half of stretch, and the dynamic peak frequency by measuring the shortest inter-impulse interval during the dynamic phase of stretch.

To change the bathing solution, the solution in the bath was withdrawn and a new solution was introduced using a syringe. This was repeated 4–6 times, which took 2–3 min. The total volume of the bathing solution was about 0·5 ml. Because of the mechanical disturbances caused during solution change the measurement of resting tension was unreliable. The tension developed by

stretch was measured at the mid-point of the hold phase of stretch. All data were recorded on magnetic tapes.

RESULTS

Glycosaminoglycans in the capsular space

The left-hand column of Fig. 1 shows cross-sections of a cat muscle spindle stained with alcian blue at varying pH. The optimum pH for the staining appeared to be 2·5. The weaker staining at pHs above or below this suggests that the stained material

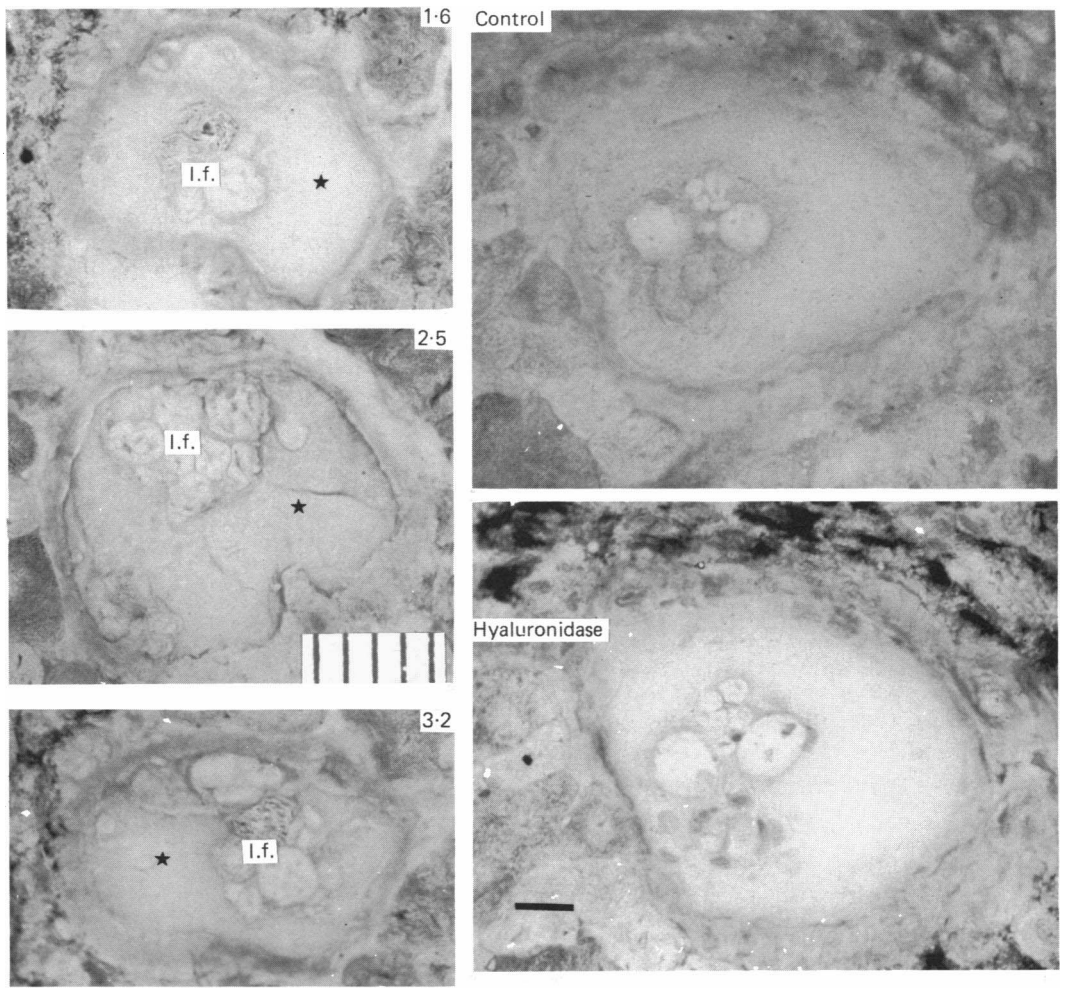

Fig. 1. Left-hand column, alcian blue staining of capsular space at various pHs, counter-stained by the periodic acid Schiff reaction. Optimum staining was obtained at pH 2·5 (middle photograph). The number in the upper right-hand corner of each photograph indicates the pH value. I.f., intrafusal fibres; star denotes capsular space. Calibration, 10 μm/division. Right-hand column, effect of hyaluronidase on alcian blue staining of the capsular space. Upper photograph, control. Lower photograph, 2·5 h after application of the enzyme to the preparation at 37 °C. Note the almost complete disappearance of staining. Formol fixation. Calibration bar, 20 μm.

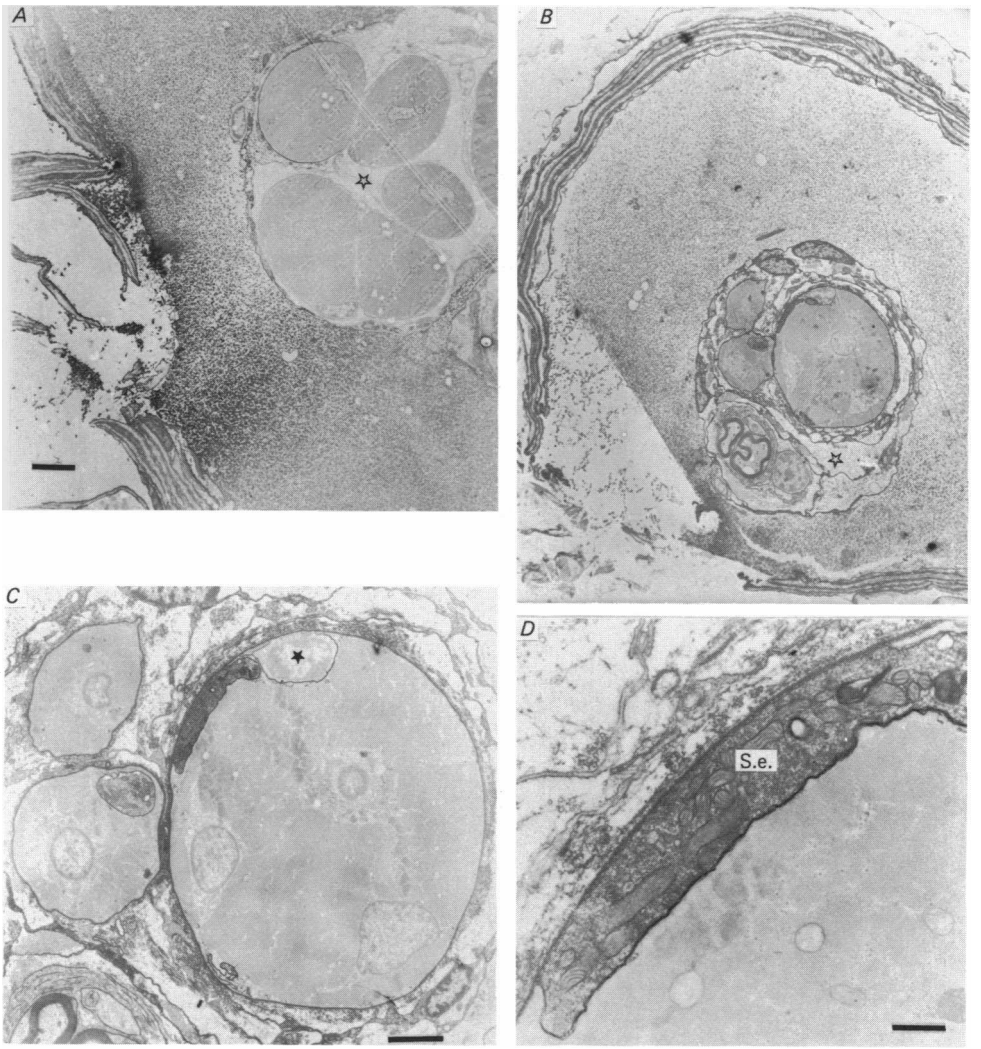

Fig. 2. Electron micrographs of muscle spindles treated with Ruthenium Red. The capsule was nicked prior to treatment with the dye. *A* and *B*, a cross-section through the nicked site from different spindles. Note the capsular space filled with darkly stained granular structures. A paucity of these granules may be seen in the space surrounded by inner capsule cells (open star). *C*, a magnified micrograph of the intrafusal fibres shown in *B*. *D*, a sensory ending (s.e.) at higher magnification. The star in *C* denotes a satellite cell. Calibration bar in *A* (5 μm) also applies to *B*. Calibration bar in *C* and *D*, 2·5 and 0·5 μm, respectively.

in the space is acid glycosaminoglycans, probably hyaluronic acid. To confirm this point the preparation was treated, prior to the staining, with a hyaluronidase. As may be seen in the right-hand column of Fig. 1 the enzyme treatment almost completely eliminated the staining. Furthermore, the result obtained by the critical electrolyte concentration technique (Scott & Dorling, 1965), using various concentrations of $MgCl_2$, showed that 0·6 M-$MgCl_2$ was sufficient to block the staining (not shown). All these results are in agreement with those obtained by Brzezinski

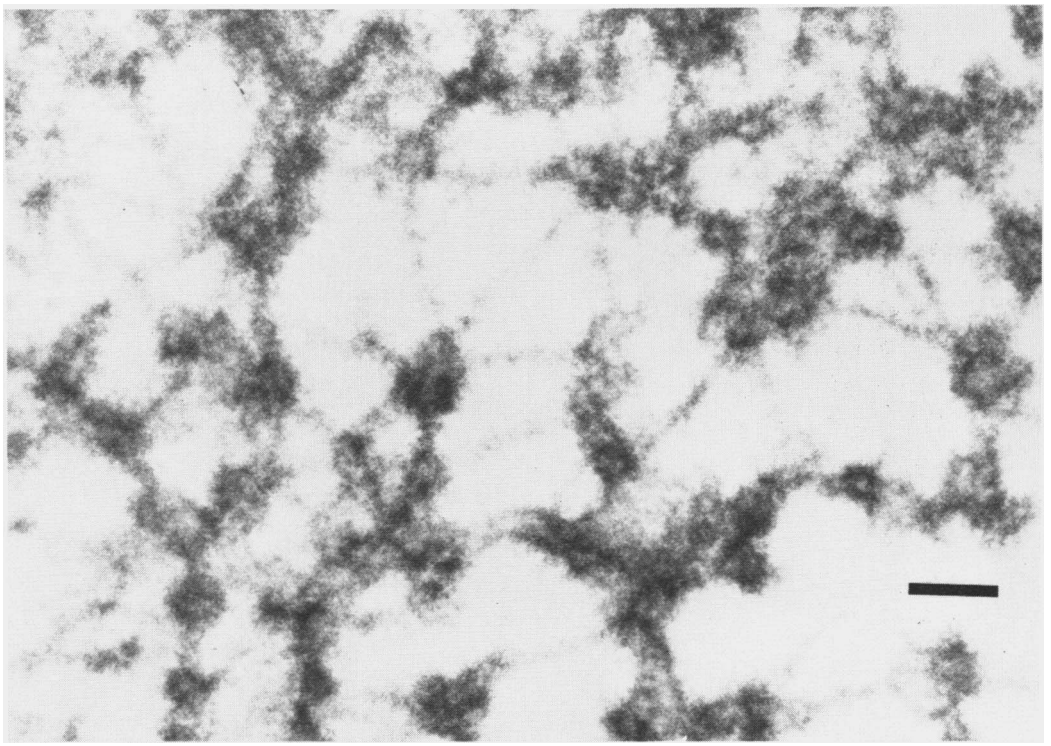

Fig. 3. Electron micrographs of a portion of the capsular space of the spindle shown in Fig. 2*B* at higher magnification. Note granular structures interconnected by thin filaments. Calibration bar, 5 nm.

(1961*a*, *b*) with guinea-pig muscle spindles, strongly indicating the presence of hyaluronate in the capsular space of cat muscle spindles.

The access of an extracellular marker, Ruthenium Red (Luft, 1966), to the capsular space was also examined. Ruthenium Red, a hexavalent cation, will bind to negatively charged hyaluronate in the capsular space if the dye penetrates the capsule. In spindles with intact capsules, however, the marker did not penetrate the capsule but gained access to the narrow space in the sleeve region through the opening of about 40 nm between the capsule and intrafusal fibres and between intrafusal fibres in this region for only a limited distance ($\sim$ 100 μm). In order to allow the dye to diffuse into the capsular space in the equatorial region the capsule was nicked using a 26 gauge hypodermic needle. As expected, the dye diffused into the space through the hole disclosing a dense network in the space which was composed of granular structures (Fig. 2*A* and *B*) interconnected by fine filaments of $\sim$ 7 nm thickness (Fig. 3). Judging from the thickness of these filaments they may represent hyaluronic acid (Fessler & Fessler, 1966), and the granular structure may consist of protein molecules. The marker also diffused into the intercellular gap between intrafusal fibres, sensory endings (s.e. in Fig. 2*D*) and satellite cells (star in Fig. 2*C*). The space surrounded by inner capsule cells (open star in Fig. 2*A* and *B*) appeared to contain less Ruthenium Red-positive substrate. The longitudinal diffusion of the dye in the

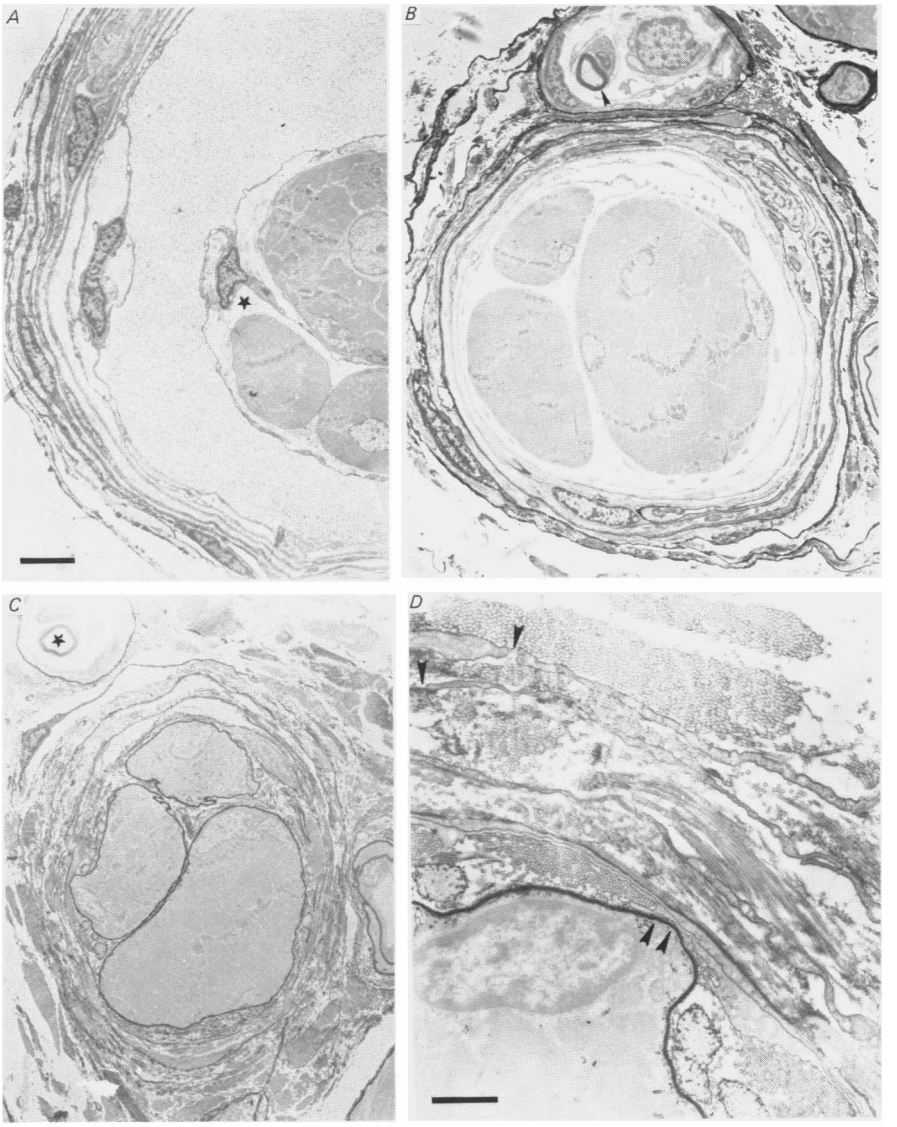

Fig. 4. Cross-section of the spindle shown in Fig. 2*B* at progressively increasing distances from the nicked site towards a pole. *A*, ~ 100 μm; *B*, ~ 500 μm; *C*, ~ 750 μm. Limited longitudinal diffusion of the dye in the capsular space is shown in *B*, where no noticeable trace of the dye was found in the space. Arrow indicates a myelinated axon. In *C*, near the end of the capsule pole, the dye appears to have diffused from the ends into the space through the gap between the intrafusal fibres and the capsule as well as between intrafusal fibres. Note the darkly stained basement membrane of intrafusal fibres. The star denotes a myelinated axon. *D*, electron-micrograph of a portion of *C* at higher magnification. The downward pointing arrowheads indicate the site of discontinuity of capsule cells. In the sleeve region the capsule cells and the intrafusal fibres were often separated only by a basement membrane as indicated by a pair of upward pointing arrowheads. Calibration bar in *A* (5 μm) applies to *B* and *C*. Calibration in *D*, 1 μm.

space towards both poles was limited; diffusion occurred for 500 μm, beyond which no trace of the dye was noticed in the space (Fig. 4*B*). The limited longitudinal diffusion is probably due to electrostatic binding of the Ruthenium Red to negatively charged hyaluronate (Fukami, 1982). Near the end of the capsule the marker appeared in the narrow space between the intrafusal fibres and the capsule, it probably diffused into the sleeve through the opening at the ends of the capsule (notice the darkly stained basement membrane of intrafusal fibres in Fig. 4*C*). In the sleeve region, even at the site where the capsule and intrafusal fibres were most closely opposed, they were separated by a 40–50 nm gap, which appeared to be filled with basement membrane (upward pointing arrowheads in Fig. 4*D*). As indicated by the downward pointing arrowheads in Fig. 4*D*, the capsule cell layer in this region often showed a discontinuity, probably representing finger-like processes of the capsule layer near the end of the capsule.

Transcapsular potential, electrical properties of the capsule and $[K^+]$ *in the capsular space*

The presence of negatively charged glycosaminoglycans in the capsular space suggests that ions may be distributed differently in the space than in the bathing solution, and that an electrical potential difference (the transcapsular potential) may be present. A glass micro-electrode filled with 3 M-KCl was introduced into the space to measure this potential. Upon penetration an abrupt potential shift was observed. The value was 10–20 mV negative inside the space (average $\pm$ S.D.; 15 mV $\pm$ 4, $n = 12$). To verify the location of the electrode tip a fluorescent dye, Lucifer Yellow, was injected through the tip of the electrode after penetration. As shown in Fig. 5 the capsular space was uniformly filled with the dye whereas intrafusal fibres appeared to be spared from the staining (arrowheads in Fig. 5). Mechanical rupture of the capsule abolished the transcapsular potential, indicating that the observed potential was developed across the capsule.

To examine passive electrical properties of the capsule, two independent glass micro-electrodes, one for passing current and the other for recording potential, were introduced into the space. All the capsules of the four spindles examined showed remarkable delayed rectification to injected current. Outward current produced less potential change than inward current of the same intensity. Input resistance measured from the slope of the hyperpolarizing current–voltage relation, and input capacitance (estimated from the time required to reach a half steady potential level) varied greatly among spindles, perhaps reflecting a wide variation of size of capsule as well as of the number of capsule cell layers (Barker, 1974; Goldfinger & Fukami, 1980). The values (input resistance and capacitance) were 3·3 M$\Omega \pm$ 3·2 and 1·0 nF $\pm$ 0·4 (mean $\pm$ S.D., $n = 4$). From the input resistance and capacitance, the specific resistance and capacitance of the capsule were roughly estimated by assuming the capsule to be a cylinder with 100 μm diameter and 850 μm length (Goldfinger & Fukami, 1980). The values for the above four spindles were 7·6 kΩcm$^2 \pm$ 7·3 and 0·5 μF/cm$^2 \pm$ 0·2 (mean $\pm$ S.D.). The above values for specific capacitance are smaller than the value for most cell membranes (1 μF/cm^2), probably due to several layers of capsule cells. The current escaping through the ends of the capsule and through the perineurium which is continuous with the capsule were neglected in the above calculation and this may also affect these calculated values.

The finding of a transcapsular potential prompted measurement of $[K^+]$ in the space. Two glass micro-electrodes, one filled with 3 M-KCl and the other filled with K^+-sensitive resin were introduced into the space for measurement of $[K^+]$ in the space. In all cases examined (six spindles), when the K^+ electrode (e_K) penetrated the capsule, a sudden positive shift of potential was observed, indicating higher $[K^+]$

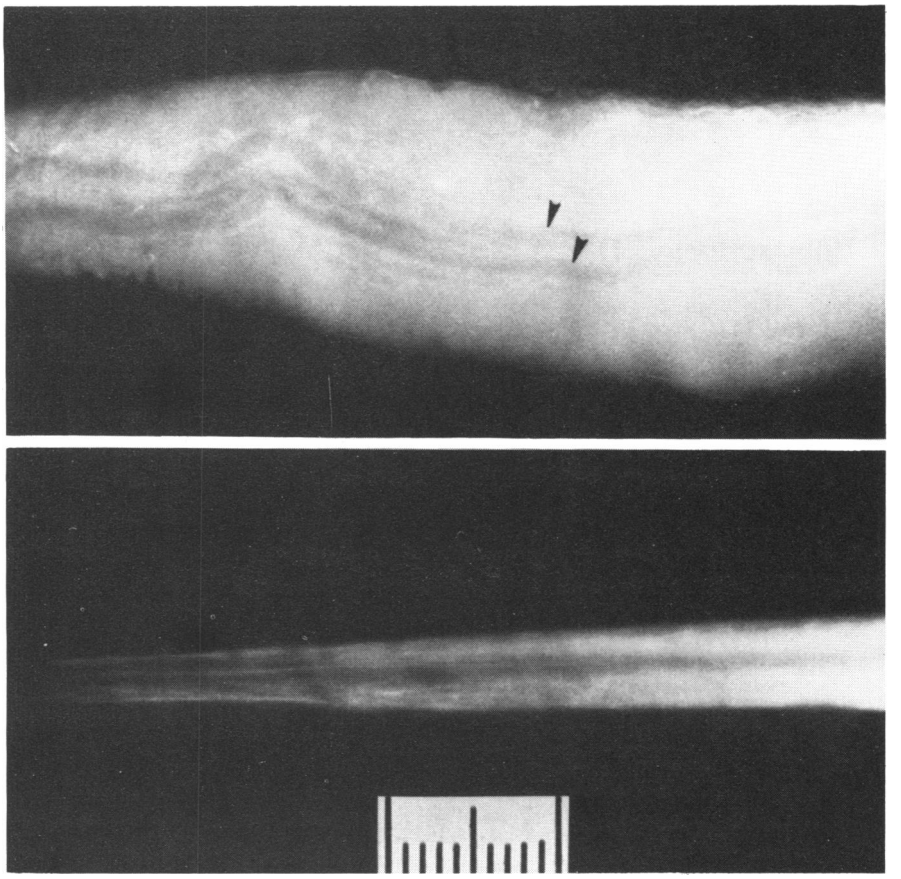

Fig. 5. Fluorescence photomicrograph of a muscle spindle (whole mount). Fluorescent dye, Lucifer Yellow, was injected into the capsular space through a glass micropipette. Intrafusal fibres (arrowheads) were spared from the staining. The upper photograph shows the equatorial region, and the lower photograph the polar region. Calibration, 10 μm/division. Transcapsular potential, -17 mV.

in the space than in the bathing solution. An example is shown in Fig. 6, where the potential electrode (e_m) was introduced into the space first (downward pointing arrowhead in the upper trace), revealing the transcapsular potential of about -10 mV (V_m). The K^+ electrode (e_K) then penetrated the capsule (downward pointing arrowhead in the lower trace). The calculated $[K^+]$ in the space was approximately 7 mM, about twice the concentration in the bathing solution (3·5 mM). The lower trace of Fig. 6 ($V_K - V_m$) indicates that ions other than K^+ are involved in the transcapsular potential. The transcapsular potential of -10 mV differs

significantly from the value calculated by the Nernst equation by assuming that only K^+ is involved (58 log 3·5/7 = −17·5 mV).

Effects of removal of hyaluronate from the capsular space

When the spindle was nicked or partially resected to expose the capsular space to the bathing solution, the background discharge and the discharge in response to stretch were slightly reduced without any significant change in tension. Hyaluronidase

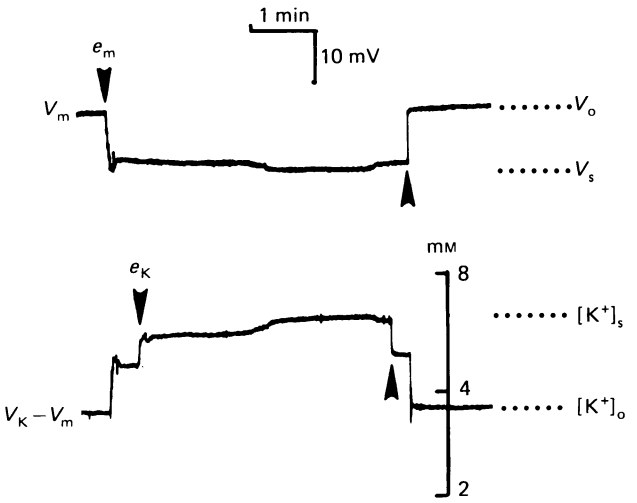

Fig. 6. Measurement of $[K^+]$ in the capsular space. The top trace represents the transcapsular potential (V_m) and the bottom trace the potential difference ($V_K - V_m$) between a K^+-sensitive resin-filled micro-electrode (e_K) and a 3 M-KCl-filled electrode (e_m). The e_m electrode was introduced into the space first (downward pointing arrowhead in top record), followed by impalement of the capsule by the e_K electrode (downward pointing arrow in the bottom trace). From the value of ($V_K - V_m$) $[K^+]$ in the capsular space was estimated to be ~ 7 mM, which is about twice that of $[K^+]$ in the bathing solution. The e_K electrode was then withdrawn (upward pointing arrowhead in bottom trace) followed by withdrawal of the e_m electrode (upward pointing arrowhead in top trace). $V_K - V_m$ was calibrated in terms of $[K^+]$ (right ordinate in bottom trace). The slope of the potentials measured by the e_K electrode against those measured by the e_m electrode in 1 and 10 mM-standard solutions was 53 mV. V_o and V_s, potential outside and inside the capsular space, respectively; $[K^+]_s$ and $[K^+]_o$, $[K^+]$ in the space and the bathing solution, respectively. Transcapsular potential, −10 to −11 mV.

(~ 10^{-4} g/ml, Sigma Type VI-S) added to the bathing solution significantly reduced, without remarkable change in tension, both background and stretch-induced impulse activities in 40–50 min. By this time the capsular space was collapsed, and hardly recognizable under a dissecting microscope (100 ×). Without the enzyme the capsular space remained for many hours. The enzyme, when applied to spindles with intact capsules, had no effect on impulse activities.

Effects of $[K^+]$

An increase in $[K^+]$ in the bath from 3·5 to 6 or 8 mM restored the impulse activities which had been suppressed by the enzyme treatment. An example is shown in

Fig. 7*A*, where the top trace of each record shows impulse activity, the middle trace tension and the bottom trace the stretch signal. On the basis of their size and response to stretch the larger impulses were taken to represent primary (Ia) ending activities and the smaller ones secondary (II) ending activities. The capsule of this spindle was partially resected before the enzyme hyaluronidase was added to the bath. In about 45 min the capsular space was totally collapsed and Ia activities (both background discharge and the response to stretch) were suppressed (Fig. 7*A*2). In comparison group II activities appeared to be less affected by the enzyme treatment. Four out of six spindles examined showed both group Ia and group II activities, while the remaining two spindles showed only Ia activities. In one spindle of the four, group II activities were more affected by the enzyme than were the Ia activities, and in the other three spindles the Ia activities were more affected than the group II activities, as is shown in Fig. 7*A*2.

Upon introduction of a 6 mM-K^+ solution the background discharge as well as the stretch-induced impulses of Ia endings was promptly (within 2–3 min taken by solution exchange, see Methods) returned to a level that was slightly above the pre-enzyme treatment level. The restored impulse activities (Fig. 7*A*3) often lasted for more than 1 h, and were reversible, as shown in Fig. 7*A*4. Reintroduction of the normal solution to the bath suppressed the impulse activities of both Ia and II axons. Suppression of group II impulses was probably due to residual enzyme action. In an 8 mM-K^+ solution impulse activities of both Ia and II axons were again restored to a slightly higher level than the level reached in 6 mM-K^+ (Fig. 7*A*5). Impulse activities of Ia axons were analysed in terms of dynamic peak frequency (top graph of Fig. 7*B*), background discharge rate (open circles in the middle graph), and the rates of discharge during the hold phase of stretch (filled circles in the middle graph). These values were plotted against time. In general, the fluctuation of stretch-induced tension during experiments did not appear to be related to the impulse activities observed. For instance, introduction of a 6 mM-K^+ solution (Fig. 7*B*) caused a slight decrease (from 2·5 to 2·0 mg) in tension whereas the impulse activities were increased during this period. Similarly, in normal solution (N) the tension was slightly increased (from 2·0 to 2·3 mg) whereas the impulse activities were significantly suppressed.

Effects of [Ca^{2+}]

The above results suggest that removing hyaluronate lowers [K^+] in the space and thus hyperpolarizes the sensory terminal membrane and reduces impulse activities. To examine this possibility further the effect of low [Ca^{2+}] was studied with four spindles. A low [Ca^{2+}] solution was prepared by mixing the normal solution, containing 1·8 mM-Ca^{2+}, with a solution containing 0 mM-Ca^{2+} and 2 mM-EGTA (1:1 or 2:1 by volume). Low [Ca^{2+}] restored the impulse activities which had been suppressed by the enzyme treatment. Fig. 8*A* illustrates example records taken from an experiment whose results were plotted in Fig. 8*B*. The capsule had been partially resected. After about 40 min in the hyaluronidase-containing solution both the background discharge and the static component of the stretch-induced response of Ia axons were abolished (Fig. 8*A*2) whereas the dynamic peak frequency showed only a slight reduction. Introduction of the low [Ca^{2+}] (1:1 mixture) solution promptly restored abolished impulse activities to a level higher (Fig. 8*B*) than the

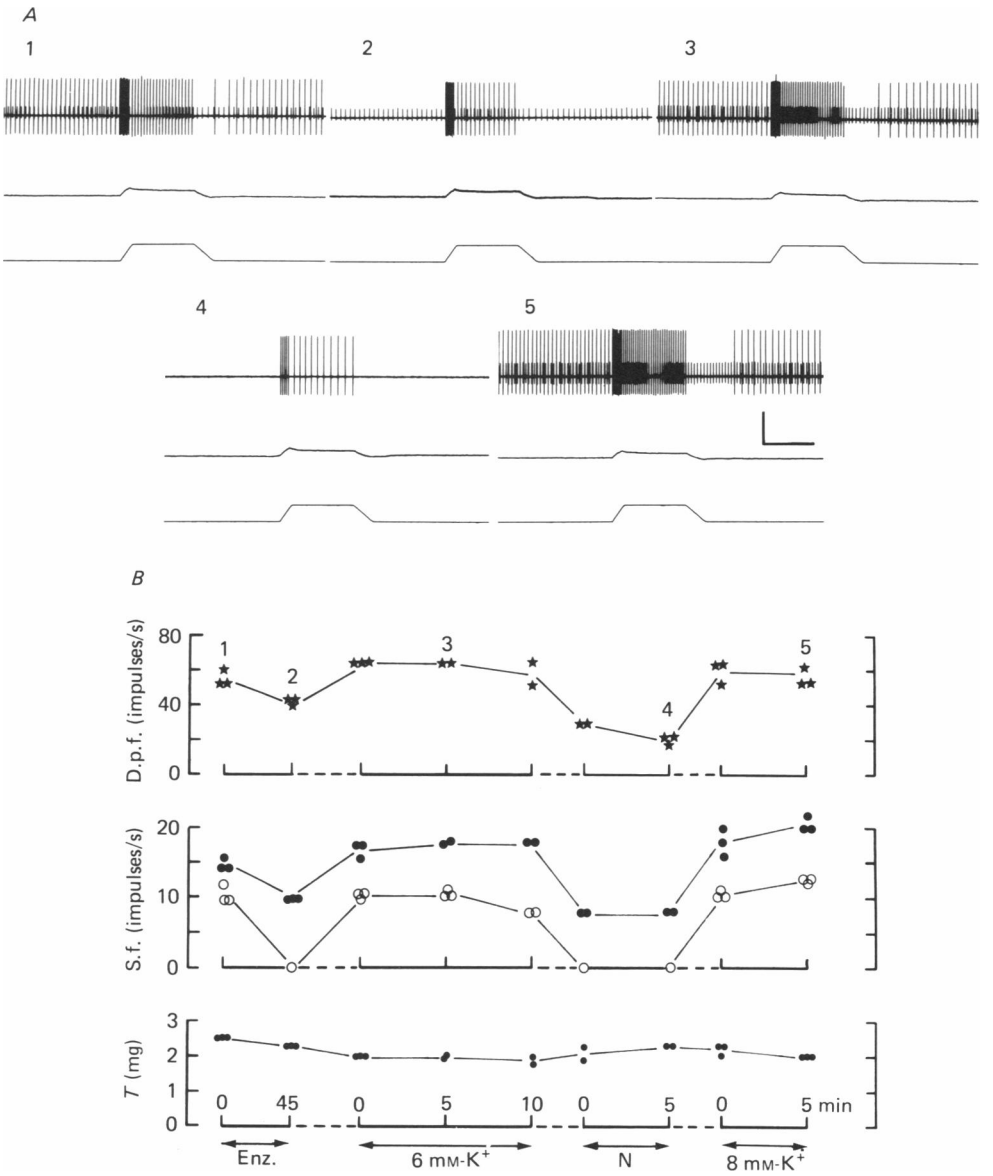

Fig. 7. The effects of 6 mM- and 8 mM-K^+ on impulse activities which had been suppressed by enzymatic removal of hyaluronate from the capsular space. *A*, sample records of data plotted in *B*. Each record is the record for the correspondingly numbered data point in *B*. In each record the top trace shows sensory discharge and the middle trace the tension developed in response to stretch (bottom trace). 1, responses just after addition of the enzyme hyaluronidase ($\sim 10^{-4}$ g/ml) to the bath; 2, responses after 45 min in the enzyme solution; 3, responses after 5 min in a 6 mM-K^+ solution; 4, responses after 5 min back in normal solution; 5, responses after 5 min in an 8 mM-K^+ solution. On the basis of their high dynamic responses and their size large amplitude impulses were taken to be those of group Ia discharge, and smaller ones group II activities. Only Ia activities were analysed and plotted in *B*. Note that there are no group II activities in record 4. Calibration: horizontal, 1 s; vertical, 12·5 mg. Stretch amplitude, 170 μm. The length of the spindle between tied ends was 13 mm. *B*, records partly shown in *A* were analysed in terms of

pre-enzyme treatment level (Fig. 8*A*3 and 4). Reversibility of the effect of low [Ca^{2+}] was in this case incomplete even after 30 min in normal solution (Fig. 8*B*). In two other spindles the reversibility was almost complete; upon introduction of the normal solution, the increased activities caused by low [Ca^{2+}] fell within a few minutes to the suppressed level. In spindles with intact capsules either low or high [Ca^{2+}] (2–3 times the normal concentration) had practically no effect within the period examined (15–20 min). It is known that the receptor potential recorded from isolated cat muscle spindles with most of the capsule removed is rather insensitive to $[Ca^{2+}]_o$ (Hunt, Wilkinson & Fukami, 1978).

Based on the above results I propose a hypothesis that the negatively charged hyaluronate ions present in the capsular space not only are responsible for the transcapsular potential but, more significantly, also depolarize the sensory terminal membrane, probably affecting the voltage-sensitive Na^+ channels in the impulse initiating site, and lower the threshold for impulse initiation (see Discussion).

DISCUSSION

Some functions of glycosaminoglycans or hyaluronic acid are ascribed to their high water retention property and viscosity. For instance, hyaluronic acid in joints acts as a lubricant, and in the C.N.S. hyaluronic acid is abundant during development and provides pathways for cell migration. Glycosaminoglycans are also thought to be involved in the development and maturation of various types of cells as well as in the process of wound healing and tissue regeneration (see Comper & Laurent, 1978; Sanes, 1983, for two reviews).

The presence of hyaluronic acid in the capsular space was first demonstrated by Brzezinski (1961*a*, *b*) in guinea-pig muscle spindles using histochemical staining techniques. His findings have been confirmed in snake muscle spindles by histochemical and electron microscopic techniques (Fukami, 1982). The present study extended Brzezinski's original findings further to cat muscle spindles by demonstrating alcian blue positive substrate, probably hyaluronic acid, the critical electrolyte concentration, hyaluronidase digestion of the substrate, and electron microscopic demonstration of a dense network in the space. Also demonstrated in the present study is that, as in snake spindles, the capsular space of cat muscle spindles is not a closed space, but communicates with the bathing solution at the capsular ends through narrow gaps (40–50 nm) between the capsule and the intrafusal fibres and between intrafusal fibres. Dow, Shinn & Ovalle (1980), using horseradish peroxidase as a marker, demonstrated that the polar region in rat muscle spindles is also leaky and open-ended at the capsular ends.

The transcapsular potential of 15 mV negative inside the space and higher [K^+] in the space are probably due partly to the negatively charged hyaluronate present

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the dynamic peak frequency (d.p.f., top graph), the static discharge rate (filled circles in middle graph), which was determined during the last half of the hold phase of stretch, the background discharge rate (open circles in middle graph) and tension (bottom graph), which was measured at the mid-point of the hold phase of stretch. Note the compressed time-scale during the enzyme (enz.) treatment. N, normal solution.

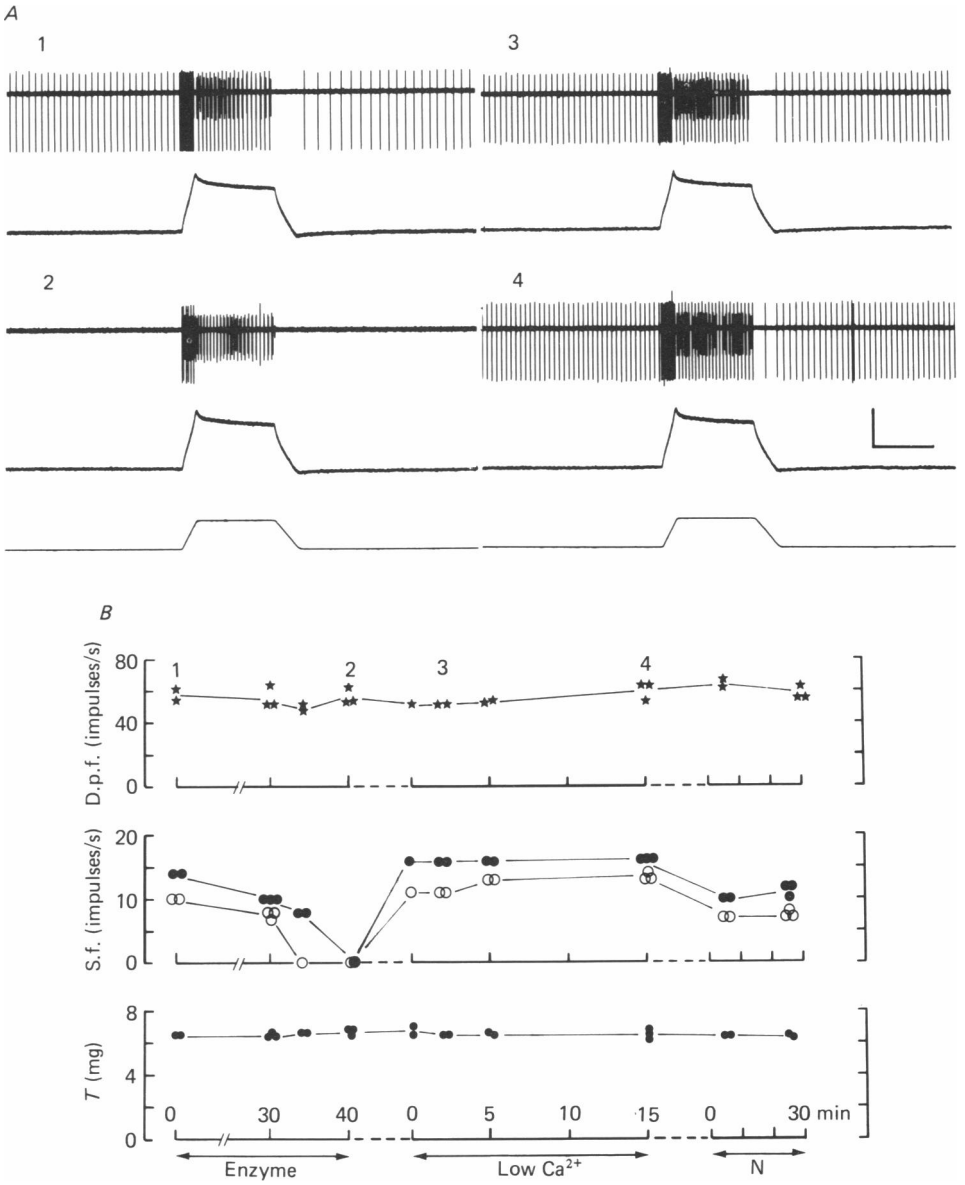

Fig. 8. Effects of low [Ca^{+}] on impulse activities which had been suppressed by enzymatic removal of hyaluronate from the capsular space. *A*, representative records of correspondingly numbered data points in *B*. In each record the top trace shows impulse activities, and the lower trace tension; the very bottom trace shows the stretch signal. 1, responses just after addition of the enzyme hyaluronidase ($\sim 10^{-4}$ g/ml) to the bath; 2, responses after 40 min in the enzyme solution; 3, responses 2 min after introduction of the low [Ca^{2+}] solution (1:1 mixture of normal solution and the solution containing 0 mM-Ca^{2+} and 2 mM-EGTA); 4, responses after 15 min in the low [Ca^{2+}] solution. In all records the stretch-induced impulse response appeared to be composed of large amplitude Ia impulses and small amplitude discharge probably from two group II axons (see record 2). Stretch amplitude, 500 μm. The length of the spindle between tied ends was 15 mm. Calibration: horizontal, 1 s; vertical, 6·3 mg. *B*, plot of records partly shown in *A*. The dynamic peak frequency (d.p.f., top graph), the static discharge rate during the hold phase of stretch (filled circles, middle graph), the background discharge rate (open circles, middle graph) and tension (bottom graph) are plotted against time. N, normal solution. S.f. (middle ordinate), static frequency.

in the space. The above findings suggest that a steady current flows out of the capsule and into the openings at the ends of the capsule. However, since nicking or partial resection of the capsule caused only a slight decrease in impulse activities, its immediate functional significance is dubious. (The steady current estimated from the input resistance of the capsule and the transcapsular potential is of the order of 10^{-6} A/cm^2. I attempted to confirm this predicted current using the vibrating probe technique. In two out of twelve snake spindles, three out of ten rat spindles and one cat spindle examined steady current flowing out of the capsule (up to 10^{-6} A/cm^2) was detected. In the rest of the spindles tested either no steady current or only a hint of such current was observed. The reason for inconsistent results is not clear.) In contrast, enzymatic removal of hyaluronate from the space resulted in, without any remarkable change in tension, total collapse of the capsular space and a significant reduction of impulse activities. The simple explanation for the recovery of suppressed impulse activities by increased [K^+] or by lowered [Ca^{2+}] would be that the enzymatic removal of hyaluronate from the space would lower [K^+] in the space, leading to hyperpolarization of the sensory terminal. However, hyaluronic acid also strongly binds Ca^{2+} (Comper & Laurent, 1978) and the enzymatic treatment of the space would remove not only excess K^+ but also Ca^{2+}; these two ions exert opposing effects on neuronal excitability. In addition, hyaluronic acid, together with some other acid (sulphated) glycosaminoglycans, may be bound to the plasma membrane of the sensory ending and extend into the extracellular space as a cell coating. Hyaluronidase removes not only hyaluronate but also to some extent other plasma membrane-associated acid glycosaminoglycans (Kiang, Crockett, Margolis, & Margolis, 1978; Margolis & Margolis, 1979), causing an increase in the surface charge (or hyperpolarization) of the sensory ending. The observed effect of the enzyme treatment may be the sum of these multiple effects.

The fact that glycosaminoglycans and proteoglycans bind significant amounts of Ca^{2+} (see Comper & Laurent, 1978, for a review) suggests that hyaluronate acts as a Ca^{2+} buffer and regulates Ca^{2+} activities in the capsular space.

The paucity of Ruthenium Red positive substrate in the capsular space surrounded by inner capsule cells (inner capsular space, marked by a star in Fig. 2*A* and *B*) is in common with the finding on snake spindles (Fukami, 1982). The paucity is certainly not due to the inner capsule cells acting as a diffusion barrier; the basement membrane of the intrafusal fibres was darkly stained with the marker and the marker also diffused into the intercellular gaps between the intrafusal fibres, sensory endings and satellite cells (Fig. 2). The substrate in the inner capsular space may be either neutral glycosaminoglycans or fluid containing less hyaluronate.

Responses to stretch of developing muscle receptors (including spindles) in the hind limb of new-born kittens has been demonstrated to be totally phasic, and the tonic component gradually appears later after birth (6–10 days post-natally; Skoglund, 1960). The phasic response may be due at least in part to the poorly developed capsular space of muscle spindles at birth (Y. Fukami, unpublished observation) and thus lack of hyaluronate. In the rat the capsular space starts to appear 5 days after birth (Barker, 1974). Since hyaluronate retains a large amount of water (up to 10^3 times its unhydrated volume; Comper & Laurent, 1978) its accumulation in the capsular space certainly contributes to the development of the space and may be related to the appearance of the tonic component of the response.

Since hyaluronate is thought to be involved not only in facilitating cell migration but also in terminating cell migration and triggering cell differentiation and maturation (see Sanes, 1983, for a review) it is also possible that hyaluronate in the capsular space is acting as an inhibitor for the development of intrafusal fibres, leaving them at a relatively primitive (myotube) stage.

I thank Drs C. Rovainen and R. S. Wilkinson for comments on the first draft of this paper and Drs L. F. Jaffe, W. J. Betz and J. H. Caldwell for kindly providing their vibrating probe facilities for my use. This work was supported by NIH Grant NS–19661 and by the Muscular Dystrophy Association (Jerry Lewis Muscular Research Center, Washington University).

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