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ADAPTATION OF THE GENERATOR POTENTIAL IN THE CRAYFISH STRETCH RECEPTORS UNDER CONSTANT LENGTH AND CONSTANT TENSION

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SUMMARY

1. Generator potentials were investigated in stretch receptors of crayfish after abolishing spike potentials with tetrodotoxin.

2. The time courses of the decline of generator potential (generator adaptation) were almost the same in the slowly and rapidly adapting receptors.

3. The time courses of the tension changes after suddenly stretching the receptor muscles did not differ much between the two receptor types.

4. The amplitudes of generator potential per unit stress or per unit strain in the receptor muscle were roughly the same in the two receptor types.

5. By comparing generator adaptation under length-clamp and tension-clamp in the slowly adapting receptors, it was suggested that roughly 70 % of the generator adaptation could be explained by a simple visco-elastic property of the receptor muscle, when observed for 1 sec after the beginning of the stretch.

6. It was concluded that the marked differences in the receptor adaptation between the two receptor types were attributable to the differences in the properties of spike generating membrane rather than to the properties of the generator potentials.

7. In each type of receptor, both the generator adaptation and the adaptation of spike generating mechanisms contributed to determining the whole rates of receptor adaptation. In the slowly adapting receptor, however, the generator adaptation seemed more important, while in the rapidly adapting receptor the spike generating mechanisms seemed more important.

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INTRODUCTION

In a previous paper (Nakajima & Onodera, 1969) it was shown that adaptation in the spike-generating membrane (spike adaptation) was markedly different between the slowly and rapidly adapting stretch receptors of crayfish. This paper will deal with problems of adaptation of the generator potential (generator adaptation). It will be shown that there are almost no differences in the generator adaptation between the two receptor types.

The mechanical factors of generator adaptation will also be analysed. By comparing the generator potentials under constant length and under constant tension, we could estimate how far a simple visco-elastic property of the receptor muscle could account for the generator adaptation. A preliminary account was published (Nakajima, 1964).

METHODS

The preparation and the general experimental procedure were the same as described in the previous paper (Nakajima & Onodera, 1969).

Method 1. *Recording of generator potentials under constant length.* Figure 1 is a schematic diagram of the arrangement. A stretch receptor organ was dissected out with tiny pieces of carapace attached to both ends of the receptor muscle. The pieces of carapace were gripped by forceps. The latter were connected to galvanometers through a lever system. The slider, which held the shaft of the forceps, transformed the circular movement of the galvanometer into a straight line movement of the forceps. Two sets of galvanometers and power amplifiers of the same characteristics were positioned at the left and right side of the system. In each experiment the gain of the amplifiers was adjusted so that a same square pulse input produced no movement of the neurone, which was not necessarily located exactly at the centre of the muscle.

The length of the muscle was recorded by a photo-electric transducer, consisting of a photocell, a square mask, a light source and a vane; the vane was in direct continuity with the left-hand forceps. The total lengthening of the muscle, i.e. the sum of displacements of both pairs of forceps, will be referred to as 'elongation' or as 'stretch'. The relation between the photocell output and the total elongation was determined after each experiment by measuring the length of the muscle under the microscope, and revealed sufficient linearity, because the ratio of the movements of the left- and right-hand forceps was constant: the error was in most cases within 30 μ, but sometimes 50 μ. Generator potentials were recorded with a micro-electrode inserted into the soma.

Method 2. *Recording of impulse frequency by extracellular electrodes and recording of muscle tension under constant length.* Stretching was performed by an arrangement similar to that of Method 1, except that the right-side end of the muscle was immobile, and the carapace was attached by a fine silver wire to a glass tube, which was fixed to the anode pin of a mechano-electronic transducer (Toshiba version of RCA 5734). The stretch was effected by displacing the left-side carapace only, resulting in a more accurate measurement of the lengthening. Impulse discharges were recorded from the central end of the nerve bundle, which was lifted up into the air by fine platinum-wire electrodes.

Method 3. *Recording of generator potentials under length- or tension-clamping.* Figure 2 shows the arrangement. The principle was to keep the output voltage from a mechano-

electronic transducer or from a photo-electric transducer constant by a feed-back amplifier and a servo-motor (Gordon, Huxley & Julian, 1966). The muscle, without carapace, was attached to two small mylar sheets by cellulose tape (Sellotape). We are indebted to Dr M. Endo for indicating the usefulness of 'Sellotape' in this respect. Each sheet was cemented to an L-shaped Pyrex glass tube (1·5 mm diameter). The left glass tube was connected to a tripod, which was fixed to a galvanometer, the length of the tripod from the centre of rotation to the point of attachment of the tube being 80 mm. The right-hand glass

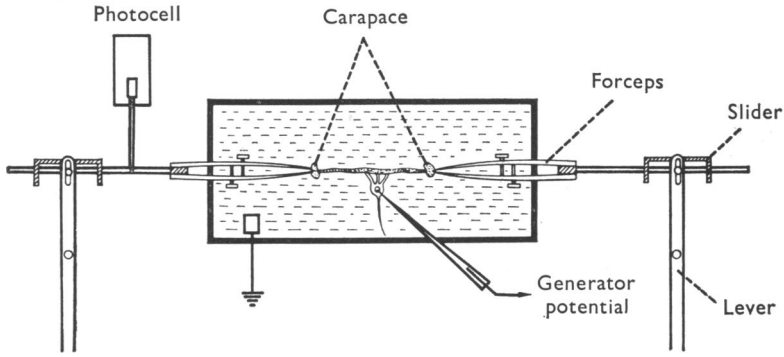

Fig. 1. Diagram of arrangement for applying constant-length stretches (Method 1). See the text.

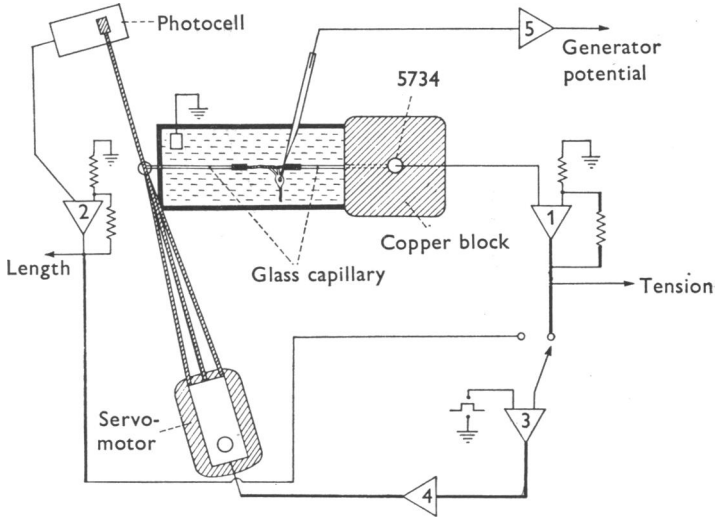

Fig. 2. Diagram of applying length- or tension-clamp (Method 3). See the text.

tube was cemented to the anode pin of a Toshiba version of RCA 5734, which was thermally stabilized by mounting it in a large copper block. The sensitivity of the transducer with the glass tube attached was about 1·5 V/g, with a natural frequency of about 200 c/s. The length of the muscle fibre was recorded by a photo-electric transducer. Because of the circular movement of the galvanometer, the output from the photo-electric transducer was not linearly related to the length of the muscle. However, we did not correct this non-linearity because the error from this source was below 0·7 % within the extent of the elongations

applied. The sensitivity of the transducers was affected by the magnetic field from the servo-motor. Therefore, calibration had to be done after each experiment with the same layout as that during the experiment.

The output from the mechano-electronic transducer was fed into the operational amplifier 1, and the output from the photoelectric transducers into the operational amplifier 2. The output of either operational amplifier was connected to the main feed-back amplifier 3. The bandwidth of amplifier 3 was reduced by an RC network so that the upper 3 db frequency was about 0·7 c/s. This determined the over-all open-loop frequency response, as well as the main phase-shift, and served to stabilize the feed-back. The galvanometer was a moving coil instrument from a pen recorder. Because the hair spring was not omitted, a 1 mA current produced an elongation of about 2 mm in the muscle; this intensity of current was supplied to the galvanometer with 0·4 mV input to the amplifier 1.

Assuming that a stretch of the muscle by 2 mm produces 10 mg tension at an initial phase of the stretch, this would produce about 15 mV at the output of 5734. This control system is equivalent to over-all voltage feed-back with an open-loop gain of about 40 (15 mV/0·4 mV). If the mechanical impedance of the muscle decreases roughly to about half, 1 sec after the beginning of stretch, the open-loop gain of the equivalent amplifier would decrease to about 20. This means that the tension could be clamped within a 3 % error. The command voltage pulses were passed through a low-pass filter, so that changes of tension and length had a rise time of about 40 msec. Too rapid application of stretch dislodged the micro-electrode; this was perhaps caused by the inertia of the tissues.

The muscle was attached to the right-hand mylar sheet about 100–200 μ from the cell body, while the left-hand muscle length was 1–2 mm. With this procedure, the sarcolemma of the muscle fibres must have been damaged at least at the points of attachment to the mylar sheets. Young's modulus of the receptor muscle was by about 30 % less than that of the intact muscle. Nevertheless, the time course of the generator potential was not very different from that obtained by Method 1, in which the sarcolemma was thought to be intact.

Generator potentials were recorded by an intracellular micro-electrode. The electrode was made flexible by the method of Freygang, Goldstein & Hellam (1964). When inserting the micro-electrode, a small glass pedestal was placed beneath the nerve cell to facilitate the impalement. After the impalement, the pedestal was lowered lest it should disturb the tension measurement. Presence of the micro-electrode inside the soma did not affect the tension–length relationship of the muscle: it was unchanged after the electrode was withdrawn.

This type of experiment was done only on slowly adapting receptors In the case of the rapidly adapting receptor, the muscle seemed to deteriorate quickly after being detached from the carapace.

RESULTS

Comparison of generator potential in slowly and rapidly adapting receptors

Figure 3 shows responses of receptor neurones during stretches of the receptor muscles. Method 1 was used. The upper trace represents the intracellularly recorded potential, and the lower the elongation of the muscle. In the slowly adapting cells (A_1) spike discharges continued as long as the stretch was maintained (only the foot of repetitive spike potentials was recorded at this amplification). On the other hand, in the rapidly adapting neurone (B_1) the discharges ceased at 7·8 sec despite the presence of a maintained stretch. A larger or smaller stretch than in B_1 curtailed the duration of the discharges.

Records A_2 and B_2 of Fig. 3 are generator potentials obtained after the

spike potential had been abolished with tetrodotoxin (2×10^{-7} g/ml.). It is seen that the amplitudes of the generator potentials declined first rapidly, then very slowly. But the time courses of the declines were almost identical in both receptor types.

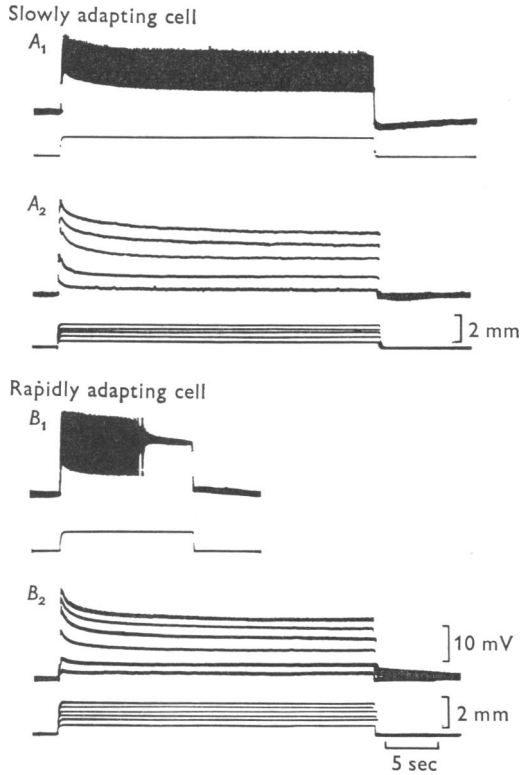

Fig. 3. Spikes (A_1 and B_1) and generator potentials (A_2 and B_2) of slowly (A) and rapidly (B) adapting neurones evoked by constant stretches. (Method 1). Lower traces show the elongation of the muscle. The resting length of the receptor muscle, which corresponds to the base line of each lower trace, was 3·8 mm in A, and 5·5 mm in B. A_1 and B_1: in normal saline. A_2 and B_2: after application of tetrodotoxin (2×10^{-7} g/ml.).

Controversial results have been reported on the effect of tetrodotoxin on generator potentials: (1) no effect (Loewenstein, Terzuolo & Washizu, 1963), (2) reduction (Nishi & Sato, 1966), and (3) increase of generator current (Albuquerque & Grampp, 1968).

We could not obtain a consistent result on this point, because it is necessary to study this on small generator potentials; otherwise, the generator potential would be contaminated by a subthreshold response of the electrically excitable membrane component (Grundfest, 1957, 1967), which is expected to be affected by tetrodotoxin. At least part of the controversial results seem to derive from this cause.

There were wide individual variations in the time courses of the generator potential. In Fig. 4 we compared slopes of the generator potentials in

the slowly and rapidly adapting receptors. The slopes differed according to the amplitudes of the generator potential, so that in making Fig. 4 the following procedure of interpolation was adopted. From the family of curves like that shown in Fig. 3, the slopes of the generator potentials at 1 sec were read off, and plotted against the voltage of the generator potential. From this graph the slopes at convenient arbitrary voltages of 10, 20 and 25 mV were determined and were shown in the upper graph

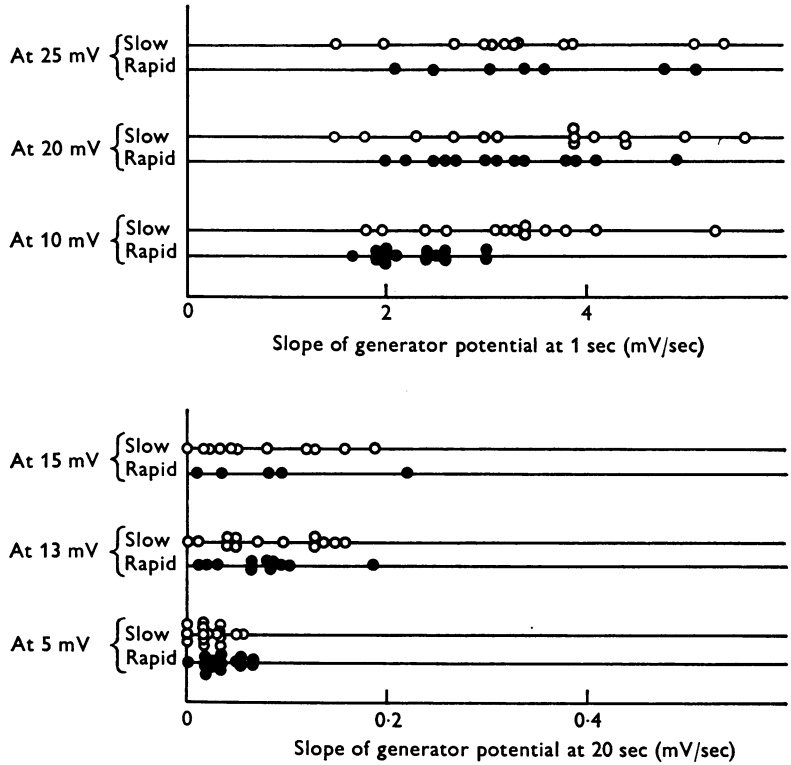

Fig. 4. Comparison of the slopes of the generator potential at 1 sec and at 20 sec after the beginning of constant stretch. The upper graph shows the slopes at 1 sec and the lower those at 20 sec after the beginning of the stretches. In many cases values interpolated from the generator potentials of neighbouring amplitudes are shown. (See the text for the explanation of the procedure of interpolation.)

of Fig. 4. A similar procedure was used at 20 sec and shown in the lower graph of Fig. 4. It can be seen that no essential difference in the slopes existed between the two receptor types. This result is contrary to that of Eyzaguirre & Kuffler (1955).

Another way of looking at the generator adaptation would be to estimate the ratio of the amplitude of generator potential at the steady state (20 sec) to that at an early phase (0·3 sec). The values of these ratios dif-

fered according to the amplitudes of generator potential, so that calculation was made only for the generator potential with an amplitude of 20 mV at 0·3 sec. The same procedure of interpolation, as explained in connexion with Fig. 4, was adopted. The ratios were $0{\cdot}39 \pm 0{\cdot}04$ (mean ± S.E. of mean) and $0{\cdot}43 \pm 0{\cdot}02$ in the slowly and rapidly adapting cells, respectively. The difference is not significant ($P > 0{\cdot}1$). In view of the considerable differences in the morphology of the dendrite endings between the two cell types (Florey & Florey, 1955), the almost identical generator adaptation is rather surprising.

There is another interesting point in Fig. 3. As was described by Eyzaguirre & Kuffler (1955), a sudden return of the stretch to the original length produced a long-lasting after-hyperpolarization (A_1). Nakajima & Takahashi (1966) attributed at least part of this after-hyperpolarization to the 'post-tetanic hyperpolarization' (Ritchie & Straub, 1957) because a similar after-hyperpolarization was obtained by stimulating the neurone electrically, not by stretch. Fig. 3 A_2 shows that after the spike potential was abolished, the after-hyperpolarization became very much reduced. This result suggests that a large part of the hyperpolarization seen in A_1 was an after-effect of the spike activity, and not of the generator potential.

In the rapidly adapting neurone this type of after-hyperpolarization never occurred, but there was usually a long-lasting after-depolarization (Fig. 3*B*). This is in keeping with the observation that 'post-tetanic hyperpolarization' produced by electrical stimulation was never found in the rapidly adapting cells (Nakajima & Takahashi, 1966).

Comparison of muscle tension developed by stretch under constant length

Figure 5 shows examples of the tension of the receptor muscle evoked by stretches under constant length. Method 2 was used. A sudden stretch of the muscle produced a quick rise of tension, which decreased, first rapidly, then very slowly. The time courses of the decline of tension were roughly the same in the two types of receptor, although the magnitude of the tension developed by a given elongation was greater in the rapidly than in the slowly adapting. This finding is in agreement with observations by Krnjević & van Gelder (1961).

The relationship between length and tension at a fixed time after the beginning of stretch showed a non-linear curve, the incremental tension becoming larger as the length was increased (Krnjević & van Gelder, 1961; Terzuolo & Washizu, 1962; S. Nakajima & K. Onodera, in preparation). Therefore, Young's modulus of the muscle differs according to the extent of the stretch. Young's modulus was calculated by the relation

$$E = \frac{\mathrm{d}F}{\mathrm{d}l} \frac{l}{\pi r^2} \qquad (1)$$

where E is Young's modulus, dF/dl the slope of the tension–length curve, l the resting length of the muscle, r the radius of the muscle at the given stretch calculated on the assumption that the volume of the muscle did not change by stretch (Krnjević & van Gelder, 1961). Table 1 lists Young's modulus at 150 % of the resting length and 20 sec after the beginning of stretch. No statistical difference was obtained in the values of Young's modulus between the slowly and the rapidly adapting receptors ($P > 0{\cdot}1$).

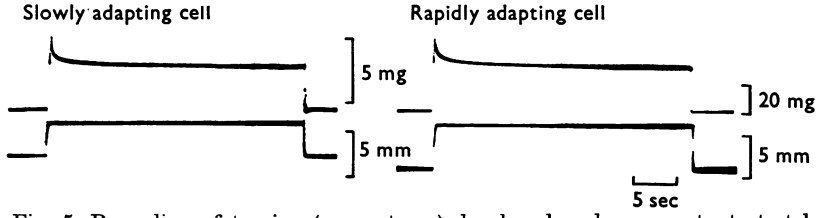

Fig. 5. Recording of tension (upper trace) developed under a constant stretch (lower trace). Resting muscle length, 3·0 mm in the slowly adapting, and 6·0 mm in the rapidly adapting receptor. Method 2.

TABLE 1. Young's modulus (E) of stretch receptor muscles at 20 sec after the onset of stretch

A. Slowly adapting receptor				B. Rapidly adapting receptor			
Cell no.	Resting length (mm)	Diameter* (μ)	E at 150 % of resting length (10^6 dyn/cm^2)	Cell. no.	Resting length (mm)	Diameter* (μ)	E at 150 % of resting length (10^6 dyn/cm^2)
440	3·5	28	0·41	443	6·5	81	0·40
450	3·7	28	1·3	444	5·0	66	0·60
455	3·0	32	0·37	445	5·5	63	1·1
457	2·9	33	0·48	447	4·6	69	0·40
459	2·9	28	0·35	449	6·0	88	0·48
460	4·3	23	2·2	456	6·0	83	0·49
Mean ± S.E. of mean	3·4 ± 0·2	29 ± 1	0·85 ± 0·31	Mean ± S.E. of mean	5·6 ± 0·3	75 ± 4	0·58 ± 0·11

* Average of the measurements at a few places over the region of 0·1–0·5 mm from the cell body.

Comparison of the sensitivity of stretch receptors

The relation between the amount of elongation (abscissa) and the amplitude of generator potential (ordinate) showed an S-shaped curve (S. Nakajima & K. Onodera, in preparation). But the curves were fairly linear over the range of generator potential of about 10–20 mV. The slope (dA/dl) of the curves over this linear range at 20 sec after the beginning of stretch is given in the third columns of Table 2A, B. The average value is 122 mV/cm in the slowly, and 68 mV/cm in the rapidly adapting receptors. Therefore, the sensitivity of the slowly adapting

receptor to a given elongation is about twice that of the rapidly adapting receptors. Because the resting muscle length is longer in the rapidly adapting receptor, the sensitivity of the receptors as referred to the normal strain, i.e. the unit elongation (dl/l), of the muscle is roughly the same. This can be seen by comparing the values of $(dA/dl) \times l$, which are given in the fourth columns of Table 2 (44 and 41 mV).

TABLE 2. Sensitivity of the stretch receptors

A. Slowly adapting receptor				B. Rapidly adapting receptor			
Cell no.	l (mm)	dA/dl (mV/cm)	$(dA/dl) \times l$ (mV)	Cell no.	l (mm)	dA/dl (mV/cm)	$(dA/dl) \times l$ (mV)
326	4·8	124	60	328	6·9	41	28
327	3·7	78	29	338	6·9	59	41
367	3·1	176	55	342	6·3	53	33
369	4·0	92	37	351	6·0	83	50
371	4·6	117	54	356	5·6	53	30
375	3·4	110	37	358	5·1	92	47
376	2·6	78	20	360	6·2	56	35
381	2·8	143	40	362	7·5	84	63
382	2·8	154	43	365	4·8	49	24
383	4·0	141	56	372	5·4	44	24
385	3·8	133	51	373	5·5	114	63
				374	6·3	84	53
Mean ± S.E. of mean	3·6 ± 0·2	122 ± 9	44 ± 4	Mean ± S.E. of mean	6·0 ± 0·2	68 ± 7	41 ± 4

l: Resting length of the receptor muscle. A: Amplitude of generator potential at 20 sec. dA/dl: Slope of linear portion of the elongation-generator potential graph at 20 sec.

If the sensitivity to unit elongation (44 and 41 mV) is divided by the Young's modulus at 150 % elongation (i.e. $0{\cdot}85 \times 10^6$ dyn/cm^2 and $0{\cdot}58 \times 10^6$ dyn/cm^2), we get the sensitivity in reference to a normal stress (total tension of the muscle divided by the transverse area). Elongation of the muscle to 150 % usually produced a generator potential of the amplitude within the linear range, i.e. 10–20 mV at 20 sec. The values obtained were 52×10^{-6} mV cm^2/dyn in the slowly and 71×10^{-6} mV cm^2/dyn in the rapidly adapting receptors. This again reveals no marked difference in the sensitivity in this respect.

However, because the mean transverse area of the slowly adapting receptor was 660 μ^2 and that of the rapidly adapting was 4420 μ^2 (from Table 1), the sensitivity of the slowly adapting receptor is about five times that of the rapidly adapting, if compared on a basis of the total tension developed in the muscle.

In summary, although the rapidly adapting receptor is less sensitive to the total elongation and to the total tension developed in the muscle, this is a consequence of the bulkiness of the rapidly adapting receptor muscle. If the same strain or the same stress was imposed upon the dendrites of

both types of neurones, the resultant generator potential would be roughly the same, despite the marked differences in the morphology of the dendrite endings (Florey & Florey, 1955).

Generator potential under length- or tension-clamping

In the muscle spindle B.H.C. Matthews (1933) and P.B.C. Matthews (1964) proposed that visco-elastic properties of the intrafusal muscle fibre were largely responsible for the production of adaptation. The simplest form of this mechanism is explained by a visco-elastic model of the receptor

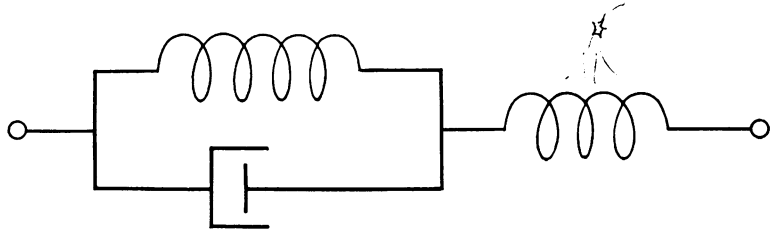

Fig. 6. Visco-elastic model of the receptor muscle. A component consisting of elasticity (a spring) and viscosity (a dash-pot) in parallel, and a component of elasticity are connected in series. The dendrite terminals are supposed to be attached to the pure elasticity of the right-hand side.

muscle (Fig. 6). If a sudden stretch is applied to the muscle, an instantaneous rise of tension is large (determined only by the right-hand elastic element, provided that we disregard a component due to the inertia of the muscle), followed by a gradual decline as the viscosity element (dash-pot) is elongated, attaining a final level, which is determined by the two elasticities in series. The dendrite terminals are supposed to be located at the elastic element of the right-hand side. Thus, if it be assumed that there are no time-dependent processes in the coupling between the tension of the right-hand elasticity and the depolarization of the dendrite terminals, the time course of the generator potential should follow the time course of the muscle tension rather than the time course of the muscle length.

The elasticity and viscosity represented by Fig. 6 are not necessarily Hookean and Newtonian ones. The above discussion holds true with non-linear elasticity and viscosity, as well as with a non-linear tension-generator potential relationship.

The structural correlates of this model were discussed in the case of the muscle spindle (P. B. C. Matthews, 1964), and the pure elasticity on the right side was assumed to correspond to the nuclear-bag region with fewer myofibrils. In the lobster stretch receptor there is a special connective tissue region around the attachment of the dendrites, but it is lacking in the crayfish stretch receptors at least under the light microscope (Alexandrowicz, 1951; Florey & Florey, 1955). Nevertheless, the paucity of contractile elements around the dendrites region can be inferred from the result that an active contraction of the receptor muscle usually produced excitation of the sensory nerves, as did a passive stretch of the muscle (Kuffler, 1954; Eyzaguirre & Kuffler, 1955).

The validity of this simple model can be tested by observing whether the generator potential and the muscle tension decline in parallel with each other under constant length. A complication arises, however, from a non-linear relation between the tension and the amplitude of generator potential (S. Nakajima & K. Onodera, in preparation). Thus, a more direct test would be to record the generator potential under constant tension.

Fig. 7 shows records of the generator potential of a slowly adapting receptor under length- (*A*) and tension-clamping (*B*). The decline of generator potential under length-clamping (*A*) was similar to that observed when Method 1 was used (Fig. 3). However, under tension-clamping (*B*) the form of generator potential approached the square-pulse shape of the tension.

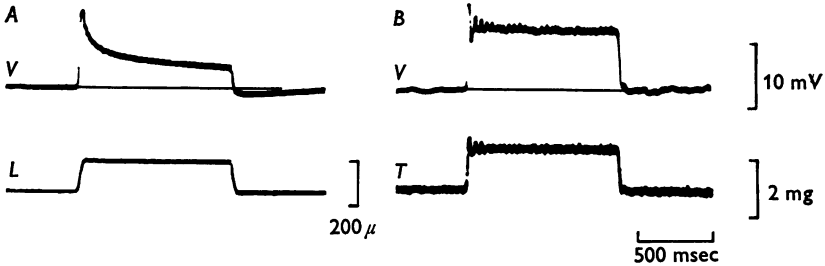

Fig. 7. *A*: a generator potential (*V*) under length (*L*)-clamping. *B*: a generator potential (*V*) under tension (*T*)-clamping. Slowly adapting neurone in the presence of tetrodotoxin (2×10^{-7} g/ml.). The resting muscle length was 1·6 mm. A conditioning stretch had been applied, so that the base lines correspond to an elongation of 0·4 mm and tension of 0·4 mg.

However, in other cells there was still some decline of the generator potential even under tension-clamping. An example is illustrated in Fig. 8. Records *A* and *C* are the generator potentials under length-clamping at fast and slow sweep speeds. Records *B* and *D* are obtained under tension-clamping. It is seen that even under tension-clamping there was a small decline of the generator potential.

Figure 8 shows that under the length-clamping (*A*) the amplitude of generator potential at 1 sec after the onset of the stretch was 64 % of that at 50 msec, whereas under the tension-clamping the corresponding figure was 94 % (*B*). Thus, under length-clamping, the value of adaptation from 50 msec to 1 sec may be taken as 36 % (100–64), and with tension-clamping 6 % (100–94). Therefore, from the total adaptation under length-clamping, about 17 % ($\frac{6}{36} \times 100$) is unexplained, while the remaining 83 % can be accounted for by the simple visco-elastic model of the receptor muscle (Fig. 6).

The reason we chose the values at 50 msec rather than at the peak of the generator potential is that the latter is very much dependent on the rapidity of stretching. Furthermore, under the tension-clamping a slight ringing at the beginning of stretch obscured the peak value of the potential.

Table 3 summarizes the result obtained by the clamping experiment. Because the adaptation behaviour of the generator potential differed according to its amplitude, we have chosen the generator potentials of the amplitude of 20 mV at 50 msec for comparison. The same procedure of

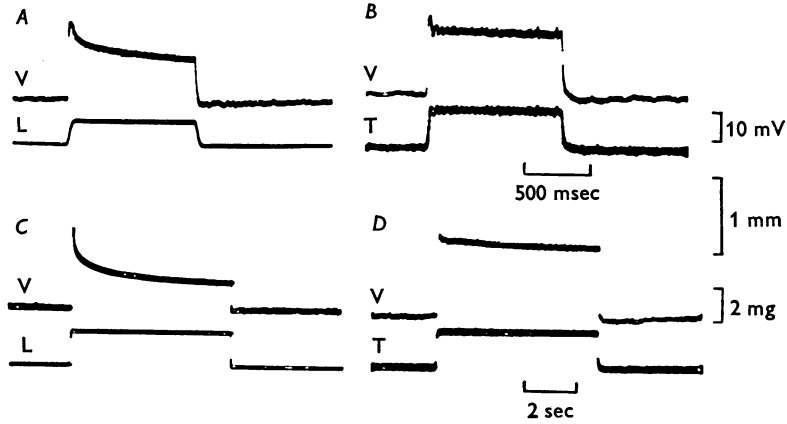

Fig. 8. *A* and *C*: generator potentials (*V*) under length (*L*)-clamping. *B* and *D*: generator potentials (*V*) under tension (*T*)-clamping. A slowly adapting cell in the presence of tetrodotoxin (2×10^{-7} g/ml.). The base lines correspond to the resting muscle length (1·8 mm), and zero tension.

TABLE 3. Comparison of generator adaptation under length-clamp and under tension-clamp in slowly adapting receptors

1	2	3	4
Cell no.	Adaptation under length clamp (%)	Adaptation under tension clamp (%)	(3/2) × 100
472	58	22	38
477	46	6	13
479	60	26	43
480	45	10	22
Mean ± S.E. of mean	52 ± 4	16 ± 5	29 ± 7

Values of adaptation are percentage decline of the generator potential from 50 msec to 1 sec. Generator potentials of the amplitude of 20 mV at 50 msec were chosen; most of the values were interpolated from the generator potentials of neighbouring amplitudes.

interpolation as already described was adopted. The Table shows that on the average, 29 % (Column 4) of the total adaptation remained unexplained, while 71 % can be attributed to the simple visco-elastic model. When observed on a slower time base and a similar calculation made on the

behaviour of adaptation from 50 msec to 6 sec, $51 \pm 7\%$ (mean $\pm$ S.E. of mean, five cells) of the total adaptation remained unexplained by the simple model.

Another interesting point about the tension-clamping experiment is that while there was an underswing of the generator potential when the length was suddenly returned to the original level (Fig. 7*A*), this underswing disappeared under tension-clamping (Fig. 7*B*). In this particular experiment, a steady stretch had been applied before application of the test stretch, so that the base line of the tension recording corresponds to 0·4 mg tension. Under this condition when the test stretch was applied and then suddenly relaxed under length-clamping (Fig. 7*A*), the tension would have imediately fallen below the base line level, i.e. below 0·4 mg, and then it would have come back to the base line level slowly. The undershoot of generator potential, thus, would be just a reflexion of these tension changes. The fact that the undershoot disappeared under tension-clamping (Fig. 7*B*) is exactly what is expected from this explanation. Thus, a large part of the underswing seen in Fig. 7*A* is simply a consequence of the mechanical properties of the muscle fibre.

DISCUSSION

Off-effect

B.H.C. Matthews (1933) observed that when the muscle spindle was stretched and then relaxed, the frequency of spike discharges temporarily fell, and then slowly came back to the original rate. Katz (1950) recorded a hyperpolarization from the nerve terminal of frog's muscle spindle associated with similar procedures. These 'off-effects' have also been observed in other mechano-receptors (Eyzaguirre & Kuffler, 1955; Florey, 1956; Loewenstein, 1956) and in several other sensory receptors (see Granit, 1955).

Eyzaguirre & Kuffler (1955) attributed the 'off-effect' at least partly to the mechanical properties of the stretch receptor muscle. Nakajima & Takahashi (1966) observed a hyperpolarization following repetitive discharges induced by electrical stimuli not by stretch, and attributed at least part of the 'off-effect' to a consequence of the spike activity (post-tetanic hyperpolarization) unrelated to mechanical properties. But these two interpretations are not mutually exclusive, and as shown in the Results of this paper either one or both can play a part in producing this effect.

For example, when started from a completely relaxed muscle, as shown by Fig. 3*A*, a larger part of the after-hyperpolarization is attributable to the 'post-tetanic hyperpolarization' because, after the spike activity was abolished, the hyperpolarization became very much reduced. But when a conditioning stretch was being applied as shown in Fig. 7 there occurred

a hyperpolarization that was to be accounted for by the visco-elastic properties of the receptor muscle fibres. This latter type of hyperpolarization seemed to have a faster time course than that due to the post-tetanic hyperpolarization, but we need more experiments to establish this point.

A small after-hyperpolarization could still be obtained even when a complete relaxed receptor was stretched and even when the spike was abolished as shown in Fig. $3A_2$. The cause of this small hyperpolarization cannot be determined at present. It might be due to a small enhancement of the electrogenic Na-pump, since the generator potential would be associated with an inward Na-flux (Edwards, Terzuolo & Washizu, 1963; Obara, 1967). The fact that this small hyperpolarization disappeared when Na was replaced with Li (Obara & Grundfest, 1968) is in keeping with this view.

These different factors could contribute to different degrees, one predominating over the other, to produce an 'off-effect' according to the conditions involved; i.e. how much conditioning stretch is applied, and how high the frequency of discharges is during the test stretch.

Generator adaptation

Several investigators have emphasized the importance of mechanical factors in determining the adaptation in mechano-receptors (B. H. C. Matthews, 1933; Loewenstein, 1956; Hubbard, 1958; Wendler & Burkhardt, 1961; P. B. C. Matthews, 1964; Loewenstein & Mendelson, 1965; Ozeki & Sato, 1965; Loewenstein & Skalak, 1966; Toyama, 1966). As a result of the length- and tension-clamping experiment, it is concluded that at a short time (1 sec) about 70 %, and at a longer time (6 sec) about 50 %, of the whole generator adaptation can be explained by the simple visco-elastic model of Fig. 6. The problem is, what would be the mechanisms of the adaptation that still remained even under tension-clamping. Obviously the model is too simple. As explained by P. B. C. Matthews (1964), it is very probable that the tissues to which the dendrites terminals are attached are not represented by pure elasticity, but contain some amount of viscosity, or that the mechanical coupling between this portion and the dendrites is not purely elastic.

One may also suppose that there might be some adaptation processes in the activation mechanisms of the dendritic membrane, i.e. a suppression of the ionic mechanisms of the generator potential analogous to the 'desensitization' of the subsynaptic membrane. But the presence of this process has never been shown unequivocally in mechano-receptors (see e.g. Loewenstein & Mendelson, 1965), although in other kinds of receptor like photoreceptor this process might be involved in producing the receptor adaptation. Fig. 7 shows that at least in this particular cell the remaining

adaptation under tension-clamping is negligibly small, favouring the view that different degrees of the remaining adaptation simply represent individual variations of the distribution of viscosity and elasticity in the muscle fibres.

Differences in adaptation between the two types of receptors

This series of papers has established that there are no differences in the generator adaptation, but there are marked differences in the spike adaptation between the two receptor types. Therefore, the different behaviours of adaptation should be attributed to the difference in spike adaptation, rather than to generator mechanisms. This conclusion disagrees with the view of Eyzaguirre & Kuffler (1955) but is in keeping with that of Krnjević & van Gelder (1961).

More specifically, in the slowly adapting neurone the soma-axonal complex can convert DC signals into long-lasting impulse trains, whereas that of the rapidly adapting receptor lacks this ability, and this difference alone explains the different adaptation behaviours of the two types of receptors. It is interesting that recently Loewenstein & Mendelson (1965), and Sato & Ozeki (1966) have found that the sensory terminal of the Pacinian corpuscle, another rapidly adapting receptor, also lacks the capability of producing long-lasting discharges.

However, it should be noted that the above view does not necessarily imply that all the receptor adaptation is simply determined by the spike adaptation (see below).

Roles of the two factors of adaptation in determining the over-all receptor adaptation

Table 4 is a comparison of adaptation between stimulation by intracellularly applied constant currents and stimulation by stretches under constant length in slowly adapting receptors. The data of the constant current stimulation were obtained from the experiments like those shown in Figs. 8 and 10 of the previous paper (Nakajima & Onodera, 1969), and those of constant stretch from experiments, in which the spike frequency was recorded extracellularly (Method 2 of this paper). Unfortunately, the data on constant current stimulation were obtained from *Orconectes* (November and December), and those on constant stretches from *Procambarus* (April and May). This table shows that under constant current stimulation the frequency of discharges declined to the level of 77 % from 0·3 to 20 sec, and under constant stretches it declined to 23 % during this period. Thus, these data suggest that of the total adaptation under constant stretch, roughly 2/7 was due to spike adaptation and 5/7 was attri-

butable to generator adaptation. In this sense it may be possible to conclude that in the slowly adapting receptor the generator adaptation is more important than the spike adaptation. This conclusion is in agreement with the view of Katz (1950) and Lippold, Nicholls & Redfearn (1960), who emphasized the importance of generator adaptation in determining the whole receptor adaptation in the muscle spindle, another example of slowly adapting receptors.

TABLE 4. Adaptation under constant current and under constant stretch stimulations in slowly adapting receptor

A. Stimulated by constant current		B. Stimulated by constant stretch	
Cell no.	Mean ratio of frequency at 20 sec to that at 0·3 sec (%)	Cell no.	Mean ratio of frequency at 20 sec to that at 0·3 sec (%)
13	73 (3)	439	26 (5)
18	86 (3)	440	27 (6)
20	70 (3)	452	20 (2)
31	85 (2)	455	21 (10)
32	77 (1)	457	21 (4)
36	70 (1)	459	20 (3)
		460	24 (2)
Mean ± S.E. of mean	77 ± 3	Mean ± S.E. of mean	23 ± 1

The values were obtained from the records, in which the impulse frequency at 20 sec lay between 15 and 30 impulses/sec. The values in the parentheses give the number of the records.

In the case of the rapidly adapting receptor, it was impossible to compare quantitatively the adaptation behaviour under constant current and constant stretch because of large individual variations. However, the fact that the spike adaptation was much more marked in this type of receptor than in the slowly adapting, while the generator adaptation was almost the same suggests that the role played by spike adaptation is far more important. In particular, the fact that a regular slow rhythm of discharge could never be encountered and that maintained spike discharges were never set up under any circumstances should be ascribed to the properties of the spike-generating membrane.

These conclusions on the relative importance of the two different adaptation mechanisms apply only under the present simple experimental conditions. We do not think that the comparison of the impulse frequencies at 0·3 sec and at 20 sec can represent all the features of receptor adaptation, which is too complex to be expressed by a single parameter. In the living animal the situation would be complicated further by the presence of motor innervation to the receptor muscle, by the inhibitory impulses impinging upon the dendrites, and by the presence of complex reflexes (Eckert, 1961; Fields, Evoy & Kennedy, 1967), and the relative importance

of the two factors would vary greatly according to the various conditions imposed upon the receptors.

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