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# TRANSDUCER PROPERTIES OF THE RAPIDLY ADAPTING STRETCH RECEPTOR NEURONE IN THE CRAYFISH (*PACIFASTACUS LENIUSCULUS*)

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SUMMARY

1. The transducer properties of the rapidly adapting stretch receptor neurone of the crayfish (*Pacifastacus leniusculus*) were studied using a two-microelectrode voltage clamp technique.

2. The impulse response to ramp-and-hold extensions of the receptor muscle typically consisted of a high frequency burst followed by cessation of impulses within a relatively short time depending on the amplitude of extension. The type of adaptation was consistent with earlier studies. The stimulus–response relationship for the impulse frequency was non-linear and had a slope in a log–log plot of 2·9.

3. When impulse generation was blocked by tetrodotoxin (TTX), (block of $Na^+$ channels) the receptor potential was extension dependent and similar to that found in the slowly adapting receptor. For small extensions there was an initial peak followed by a fall to a steady potential level. For large extensions the potential response during the ramp phase consisted of a peak followed by a constant potential level lasting to the end of the ramp. When the extension changed to the hold phase the potential fell towards a steady state. The relation between extension and amplitude of receptor potential was non-linear and saturated at $-40$ to $-30$ mV (extensions $> 15\%$ of zero length, $l_0$).

4. When potassium channels were blocked by TEA (50 mM) and 4-aminopyridine (4-AP, 5 mM) (and $Na^+$ channels blocked by TTX) the shape of the generator potential become less complex with an increased amplitude for large extensions.

5. When the receptor neurone was voltage clamped at the resting potential, extension of the receptor muscle produced an inwardly directed receptor current, the stretch-induced current (SIC). The response consisted of a fast transient phase which decayed towards a steady state. The SIC peak amplitude was dependent on extension in a sigmoidal fashion and saturated at 190 nA (extensions $> 25\%$ of $l_0$). The slope of the steepest part of the stimulus–response relation (between 10 and 20% extension) was $4{\cdot}7 \pm 0{\cdot}25$ (mean $\pm$ S.E.M.) in a log–log plot.

6. The peak amplitude of the SIC increased with increasing extension speed (ramp steepness), the relation between the slope of the ramp and current amplitude being a first order (hyperbolic) function. The amplitude of the receptor current was voltage dependent and had a reversal potential of $+16{\cdot}2 \pm 1{\cdot}8$ mV (mean $\pm$ S.E.M., 32 cells).

From the reversal potential the permeability ratio, $P_{Na}/P_K$, of the transducer permeability system was calculated to be 1·5. The $I$–$V$ curve of SIC was non-linear.

7. When the external $Ca^{2+}$ concentration was lowered the receptor current amplitude increased. This led to displacement of the stimulus–response relation towards smaller extensions. No change in reversal potential was observed.

8. The receptor current amplitude was reduced when the normal bathing solution was replaced by solutions containing mainly $Ca^{2+}$ or $Mg^{2+}$ ions. Small changes in the reversal potential for the receptor current were seen. Calculations from the current amplitudes at normal and high divalent cation solutions resulted in the following ratios: $P_{Ca}/P_{Na} = 0{\cdot}44 \pm 0{\cdot}06$ and $P_{Mg}/P_{Na} = 0{\cdot}60 \pm 0{\cdot}05$ (mean ± S.E.M., 4 cells).

9. It is concluded that the transducer properties of the rapidly adapting are similar to the slowly adapting stretch receptor neurone. The permeability ratios of the transducer membrane for different ions, $P_{Na}:P_K:P_{Ca}:P_{Mg}$, was 1:0·68:0·44:0·60. The time course of adaptation of the SIC was found to be faster and the stimulus–response relation for the SIC was steeper compared with the slowly adapting stretch receptor neurone, which could in part explain the more rapid adaptation.

## INTRODUCTION

The abdominal stretch receptor organ of crustaceans, first described by Alexandrowicz (1951), has been the subject of a number of studies regarding its function. A substantial amount of work has been done on the slowly adapting stretch receptor neurone (SRN) to characterize the transducer properties, even down to stretch-activated (SA) channels in both crayfish and lobster (Kuffler, 1954; Eyzaguirre & Kuffler, 1955; Terzuolo & Washizu, 1962; Nakajima & Onodera, 1969*a*, *b*; Klie & Wellhöner, 1973; Brown, Ottoson & Rydqvist, 1978; Edwards, 1982; Erxleben, 1989; see also Rydqvist, 1992).

In contrast to the wealth of data regarding the slowly adapting SRN, surprisingly little information is available regarding the rapidly adapting SRN. Nakajima & Onodera (1969*a*, *b*) compared the slowly and rapidly adapting SRN in the crayfish and found that the slowly adapting neurone could give sustained impulse firing at constant extensions of the receptor muscle whereas the rapidly adapting neurone ceased firing after a short while. The number of impulses fired was dependent on the level of extension. They also found that the tension response of the receptor muscle was similar in the two receptors and that the receptor potential after block of the action potentials by tetrodotoxin (TTX) differed by only a little. They concluded that the difference in adaptive behaviour is probably dependent on the so-called encoder properties, i.e. voltage-gated channels in the neurone. Such channels could either be the classical $Na^+$ and $K^+$ channels (Rydqvist & Zhou, 1989; Rydqvist & Purali, 1991; Purali & Rydqvist, 1992) generating the action potentials but also other channels like the Q-channel found in lobster stretch receptor neurones (Edman, Gesterlius & Grampp, 1987). In a recent study (Rydqvist & Purali, 1991) of the outward potassium current in the rapidly adapting SRN it was found that the kinetics of this permeability system differed in several ways compared to the slowly adapting neurone. Furthermore, using TEA and 4-AP Purali & Rydqvist (1992) found evidence that the outward rectifying and inactivating outward current is due to the presence of two potassium channel populations and that the relative

occurrence of these two channel types differs in the slowly and rapidly adapting SRNs.

However, even if the channel properties of non-SA channels do differ, it cannot be excluded that mechanotransducer properties contribute to the difference in adaptive behaviour in the slowly and rapidly adapting SRNs.

Since no detailed information is available we have now investigated the transducer properties of the rapidly adapting crayfish stretch receptor neurone. We studied the impulse response, the receptor potential using both TTX, TEA and 4-AP and the receptor current using voltage clamp. It was found that the transducer properties are in general similar to those of the slowly adapting receptor. However, the adaptive phase of the receptor current is faster and the stimulus–response relationship is steeper for the rapidly than for the slowly adapting neurone, which might contribute to the difference in adaptation.

## METHODS

### *Preparation and solutions*

The experiments were carried out on the rapidly adapting stretch receptor neurone (SRN) of the crayfish *Pacifastacus leniusculus*. No quantitative or qualitative differences regarding the transducer properties could be established between this species and the crayfish *Astacus astacus* used earlier to study transducer properties in the slowly adaptive receptor organ. The receptor neurone together with its muscle was isolated and mounted in a small chamber which could be perfused at a rate of up to 4 ml $min^{-1}$. Care was taken to dissect the receptor muscle as completely as possible and the resting lengths in this series of experiments were between 4·5 and 6·5 mm. The experiments were carried out at room temperature (20 °C). The composition of the control solution (van Harreveld, 1936) and other solutions is given in Table 1. Osmolarity measurements were made on a Roebling osmometer (Herman Roebling Messtechnik, Berlin, Germany) and all solutions were composed to give an osmolarity of $420 \pm 10$ mosmol $l^{-1}$. In some experiments the axon was pinched about 200–300 $\mu$m from the cell soma in order to minimize the error in voltage clamp due to regenerative discharges in the axon. In most experiments TTX (0·3 $\mu$M, $10^{-7}$ g $ml^{-1}$) was added to the solution to block the fast inward sodium current. This concentration effectively blocked the action potentials in the SRN (Lowenstein, Terzuolo & Washizu, 1963).

### *Recording and stimulation*

The apparatus and the methods for stimulation and recording have been described previously (Brown *et al.* 1978; Johansson & Rydqvist, 1983; Rydqvist & Zhou, 1989). The micropipettes (GC150F, Clarke Electromedical Instruments, Reading, Berks) for membrane potential measurements were filled with 3 M KCl (5–10 MΩ) and the micropipettes for injecting current were filled with 2 M potassium citrate or 3 M KCl (5–10 MΩ). The reference electrode consisted of a micropipette filled with 3 M KCl–Agar mixture and connected to an Ag–AgCl pin. The mechanical stimulations of the stretch receptor were made using the previously described pen-motor based stretching device (Brown *et al.* 1978; Johansson & Rydqvist, 1983). The ramp-and-hold stretch pulses were generated by a computer using a pCLAMP DA–AD stimulation and sampling system (Axon Instrument Inc. Foster City, CA, USA). The zero length, $l_o$, was determined from visual inspection in the dissecting microscope as the length at which the muscle was just taut after being at complete rest for several minutes. In addition, it was ascertained that no membrane potential changes were seen when small length changes were applied manually. The sampling frequency was usually set at 20 kHz, which is sufficient to acquire the fast action potentials correctly.

In a few recordings the potential and current signals were fed into a tape recorder (Hewlett-Packard 3960, band pass 0–5000 Hz) from which the data could be retrieved.

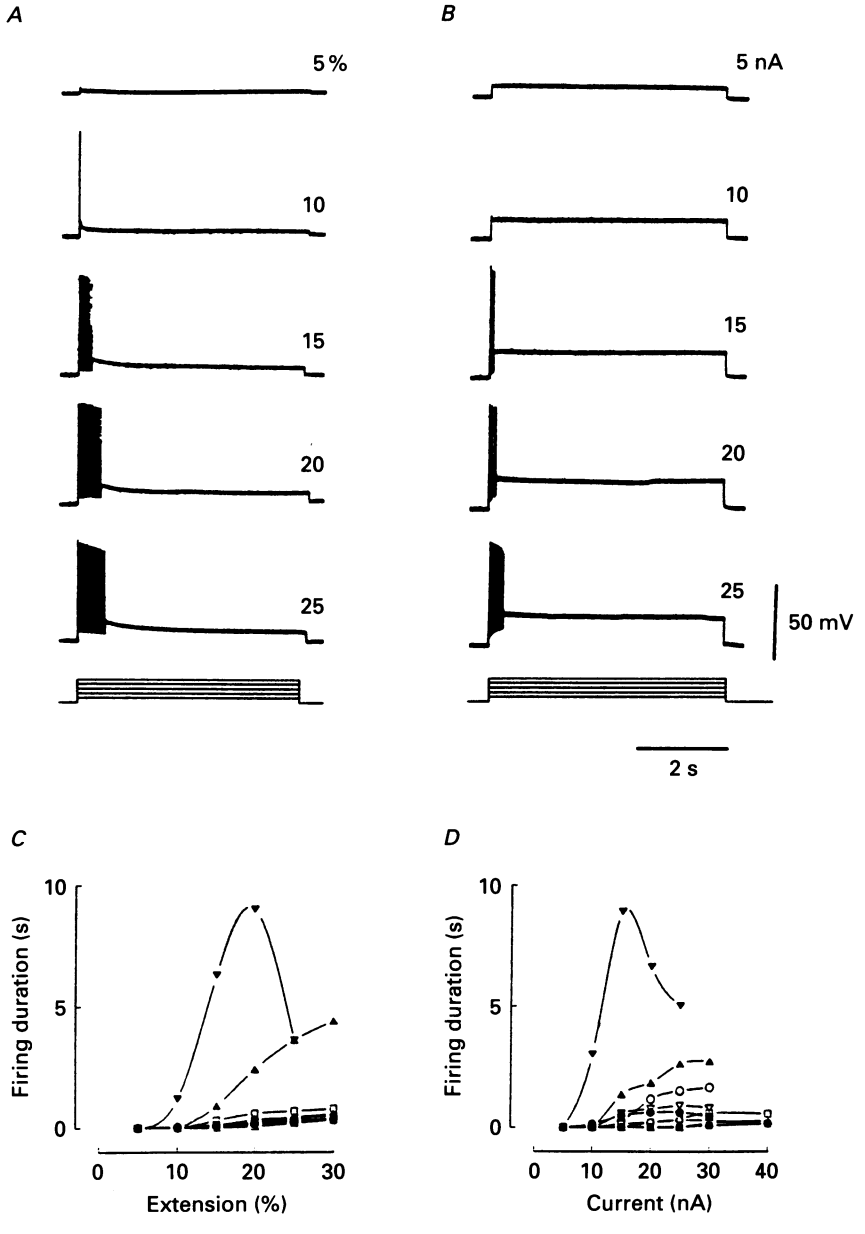


Fig. 1. Impulse responses due to mechanical and electrical stimulation (duration 5 s) from a rapidly adapting stretch receptor neurone. *A*, ramp-and-hold extensions of receptor muscle having a ramp slope of 1500% $s^{-1}$; extension amplitude indicated to the right. *B*, rectangular current steps with a rise time of less than 1 ms. The amplitude of the current indicated to the right. *C* and *D*, duration of firing *versus* extension of receptor muscle and injected current respectively for the cell in *A* and *B* (□) and 7 other cells.

RESULTS

*Impulse response*

The impulse response is the final stage of the mechano-transduction process and is also the response which has been used to characterize the adaptive properties of the

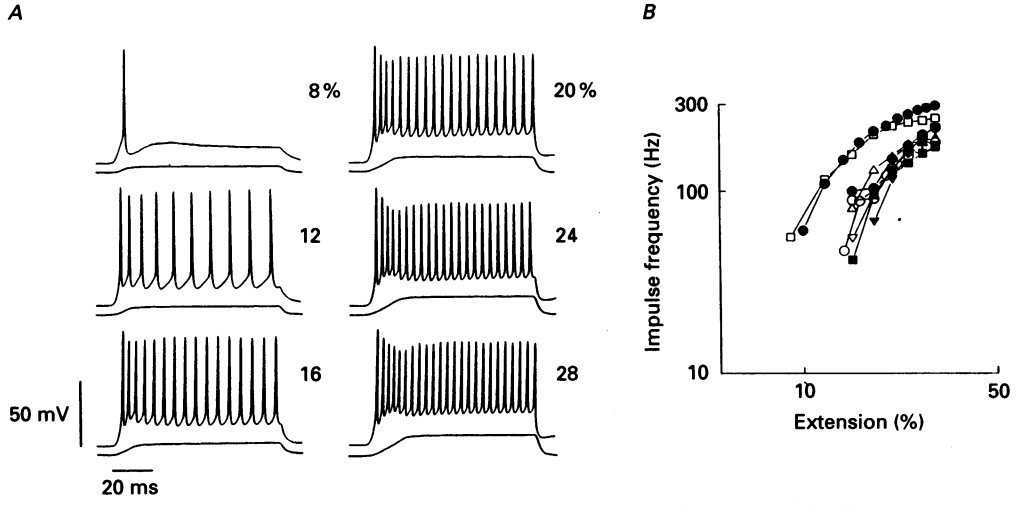


Fig. 2. *A*, impulse responses to increasing amplitudes of ramp-and-hold extensions (duration 100 ms). The slope of the ramp was 1500 % $s^{-1}$. Amplitude (%) indicated to the right. *B*, log–log plot of impulse frequency *versus* extension amplitude from cell in *A* and 8 other cells. The frequency was calculated from interspike interval about 60 ms from start of stimulus.

receptor. Figure 1*A* shows potential recordings in response to various amplitudes of extensions (duration 5 s). In this cell extensions below 5 % of zero length ($l_0$) caused only small subthreshold depolarizations at resting membrane potential whereas extensions above 5 % resulted in a train of action potentials. Both the duration and frequency (cf. Fig. 2) of the impulse train increased with increasing extension, the maximum duration being about 0·6 s in this cell. When the receptor neurone was stimulated by depolarizing current injections (Fig. 1*B*) into the cell soma, an impulse response similar to that obtained by stretch was observed. With increasing current injections both the impulse frequency and the duration of the impulse response increased. The duration of the impulse firing varied considerably between different cells (as also observed by Nakajima & Onodera, 1969*b*) but cells that had a short duration of firing when stimulated mechanically also had a short duration of firing when stimulated electrically. The behaviour of eight cells can be seen in Fig. 1*C* and *D* where the duration of firing is plotted *versus* extension and injected current respectively. In the majority of these neurones the impulse discharge stopped within 1 s. However, two neurones fired for longer periods (4 and 8 s) of time and for the cell that had the longest duration of firing this peaked at 8 s. Furthermore, increase of the stimulus amplitude (stretch and current) decreased the duration of impulse firing.

Figure 2*A* shows the potential responses to 100 ms extensions of different amplitudes. The impulse frequency increased with increasing amplitude of extension

and had its highest value during the ramp. During the hold phase of the extension the frequency gradually decreased with time. Another observation in this type of recording was the gradual decrease in the action potential amplitude as the stimulus strength increased, which may possibly reflect inactivation of the sodium system.

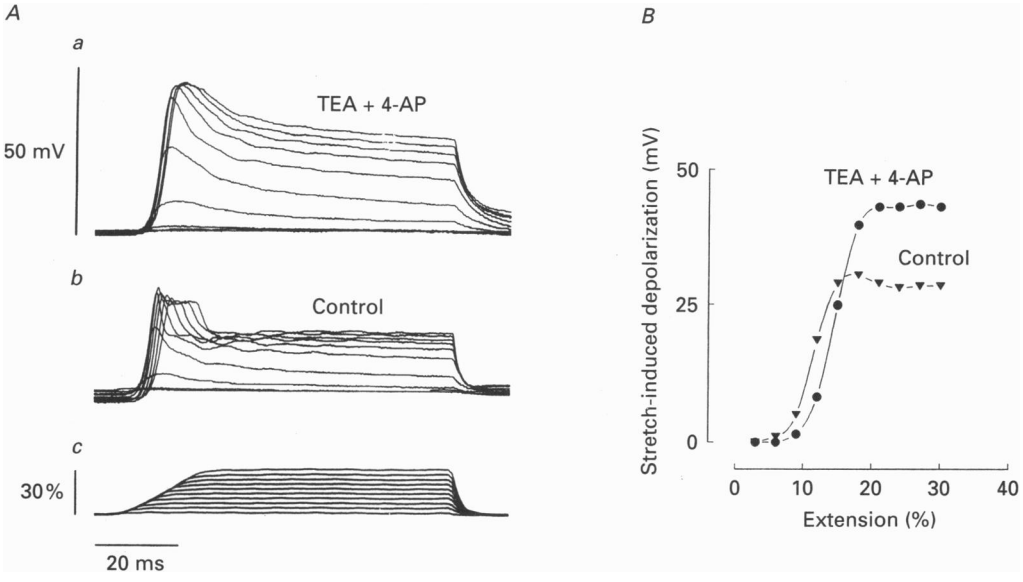


Fig. 3. Comparison of receptor potential due to ramp-and-hold extensions (3–30%, slope of ramp 1500% $s^{-1}$) in control solution and in a solution containing 50 mM TEA and 5 mM 4-AP. *A*, superimposed receptor potentials recorded in a 50 mM TEA, 5 mM 4-AP-containing solution (*a*), control solution (*b*) to ramp-and-hold extensions incrementally increased from 3 to 30% in steps of 3% (*c*). *B*, peak amplitude of receptor potentials plotted against extension for data shown in *A*. TTX 0·3 $\mu$M was present in all the solutions used.

This is supported by the fact that the first action potential had a constant amplitude in all records. In Fig. 2*B* the impulse frequency obtained from nine cells is plotted against extension amplitude. The impulse frequency was calculated from the interspike interval at about 60 ms from the start of the stimulus pulse. The relation between impulse firing frequency and extension was non-linear for the stimulus range studied and for large amplitudes the frequency increase levelled off. The mean frequency value at 30% extension was $218 \pm 12$ Hz (mean $\pm$ S.E.M., 9 cells). The mean slope of the relations in the log–log plot, as estimated from the steep (linear) part of the curves, was $2{\cdot}9 \pm 0{\cdot}2$ (mean $\pm$ S.E.M., 9 cells).

*The receptor potential*

In the presence of 0·3 $\mu$M TTX, action potentials were blocked and mechanical stimulation gave rise to a so-called receptor potential. In Fig. 3*Ab* are shown the responses to ramp-and-hold extensions from 3 to 30% of zero length ($l_0$). For small extensions the receptor potential was a simple monophasic response, whereas for extension from 18% and upwards there was a peak depolarization followed by a plateau level of depolarization which lasted till the end of the stimulus ramp. During

the hold phase the potential rapidly decayed to a steady level of depolarization similar to that observed in the slowly adapting SRN (Rydqvist & Swerup, 1991). Since $Na^+$ channels were blocked by TTX this behaviour might be due to an interaction between SA-channels and voltage-gated potassium channels; if so, block

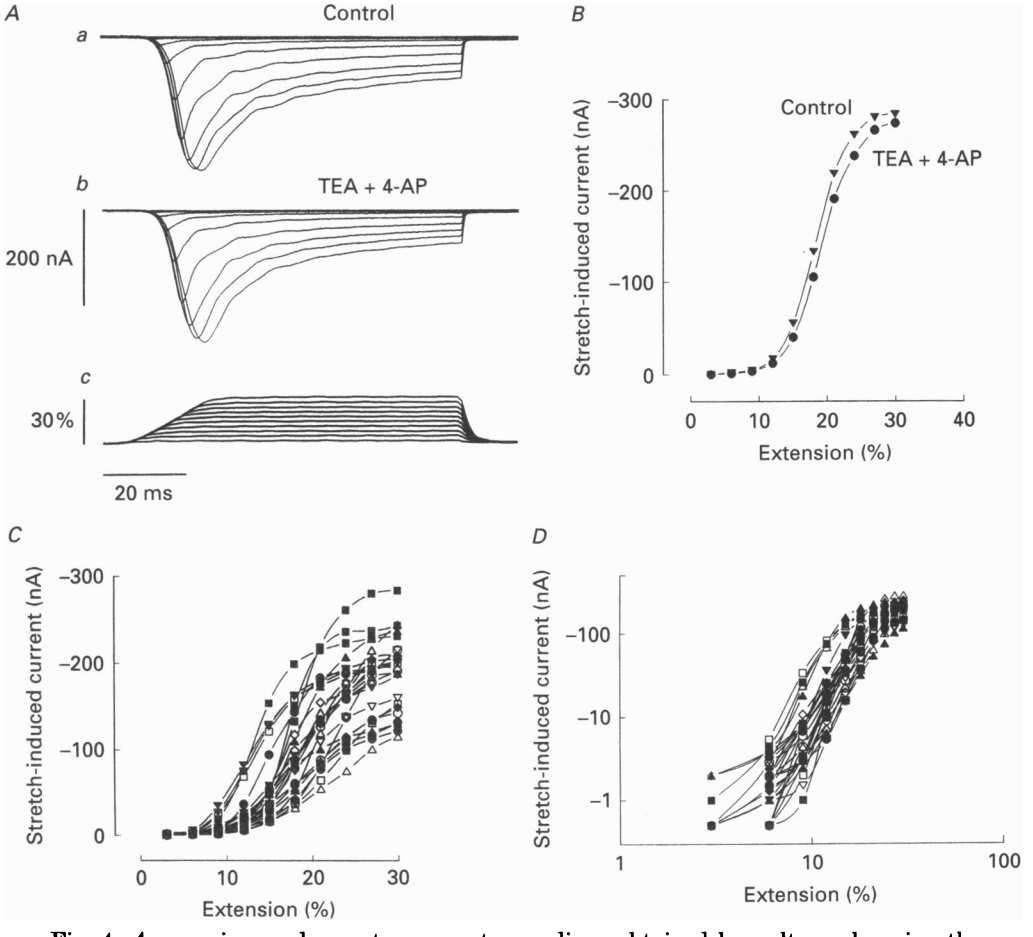


Fig. 4. *A*, superimposed receptor current recordings obtained by voltage clamping the neurone at its resting potential (−73 mV) in control solution (*a*) and at −68 mV in a 50 mM TEA, 5 mM 4-AP-containing solution (*b*). Ramp-and-hold extension (*c*) was incrementally increased from 3 to 30% in steps of 3%. The slope of the ramp was 1500% $s^{-1}$. *B*, peak amplitudes of receptor currents plotted against extension from data shown in *A*; *C*, the amplitude of the peak receptor current obtained from 28 cells is shown against extension. *D*, the stimulus–response relation of the data in *C* shown in a double logarithmic diagram (log–log plot). TTX (0·3 μM) was present in all experiments.

of these channels should abolish the plateau. This was indeed observed when the cell was exposed to 50 mM TEA and 5 mM 4-AP, which has been shown to block about 80% of the outward potassium current in the rapidly adapting SRN (Purali & Rydqvist, 1992). The plateau disappeared and the peak amplitude of the receptor response continued to increase for larger extensions. The adaptive phase become less complex and followed a smooth curve (traces in Fig. 3*Aa*). In Fig. 3*B* is plotted the

peak amplitude of the receptor potential against extension amplitude, obtained in control solution and in TEA + 4-AP-containing solution. The shape of the stimulus–response relations was generally sigmoidal, and it can be seen that there was a 40% increase in the peak amplitude for large extensions when the cell was exposed to TEA + 4-AP, which indicates that potassium channels might contribute to the reduction seen in the control solution.

*The receptor current or stretch-induced current (SIC)*

When the receptor neurone was voltage clamped at the resting potential an inwardly directed receptor current was evoked when the receptor muscle was extended (Fig. 4*A*). The receptor current consisted of an initial fast peak which decayed to a steady level in a time-dependent manner. In contrast to the behaviour of the receptor potential as seen in Fig. 3 the receptor current amplitude was only little affected by the addition of TEA + 4-AP to the bathing solution (Fig. 4*A* and *B*). This indicates that neither TEA nor 4-AP affects the mechanotransducer system *per se* (i.e. SA-channels) in agreement with what was observed by Erxleben (1989). The decay phase (or adaptation) of the SIC was studied at the resting membrane potential and the fast time constant was found to be $5{\cdot}6 \pm 0{\cdot}3$ ms (mean ± S.E.M.) in twenty-three cells as calculated from responses using extensions between 9 and 21%. This value was lower than that found for the slowly adapting neurone (*ca* 7 ms, Swerup, Rydqvist & Ottoson, 1983), but a direct comparison is difficult because of the differences in rate of extension. In one experiment (Fig. 5) it was possible to compare the fast time constant of adaptation in a slowly and a rapidly adapting SRN from the same side and segment and under identical conditions. The decay of the falling phase was considerably faster in the rapidly adapting SRN as compared to the slowly adapting SRN. This is more clearly seen in Fig. 5*B* where the responses have been plotted in a log diagram. The mean fast time constant for the decay was 7 ms for the rapidly adapting SRN and 19 ms for the slowly adapting SRN as calculated from the three stretches shown in Fig. 5. It is also obvious from the log plot (Fig. 5*B*) that the decay phase cannot be described by a single exponential. The late time course differs less for the two neurones.

In Fig. 4*C*, the peak amplitude of the receptor current in response to increasing amplitudes of stimulus from twenty-eight cells is plotted against extension amplitude on a linear scale. The stimulus–response relationship is sigmoidal. There was a considerable cell-to-cell variation, which probably originates from several sources such as the degree of pre-stretch (i.e. $l_0$), cell size, geometry of the cell and the number of SA-channels present in the cell membrane.

When the stimulus–response relationship of the receptor current was plotted on a log–log scale (Fig. 4*D*) sigmoid curves were also obtained. Thus, the stimulus–response relation cannot be described by one single power coefficient, $n$, in the Stevens power relation (Stevens 1957) as was also found in the slowly adapting SRN (Rydqvist & Swerup 1991). Different $n$ values for different parts of the curves were obtained. For small extensions $n$ is close to 1, which was also observed in the slowly adapting receptor (Johansson & Rydqvist, 1983; Rydqvist & Swerup, 1991). In all the cells studied the slope had its highest value for extension amplitudes between 10–20%. Above 20% extension, the value of $n$ gradually decreased, finally

approaching zero. The mean value of $n$ (between 10–20%) obtained from twenty-eight cells was $4{\cdot}7 \pm 0{\cdot}25$ (mean $\pm$ S.E.M.).

*Effects of varying the rate of stretch*

The amplitude of the receptor response and the adaptive behaviour were shown to depend on several factors, one of which was the characteristics of the extension. To

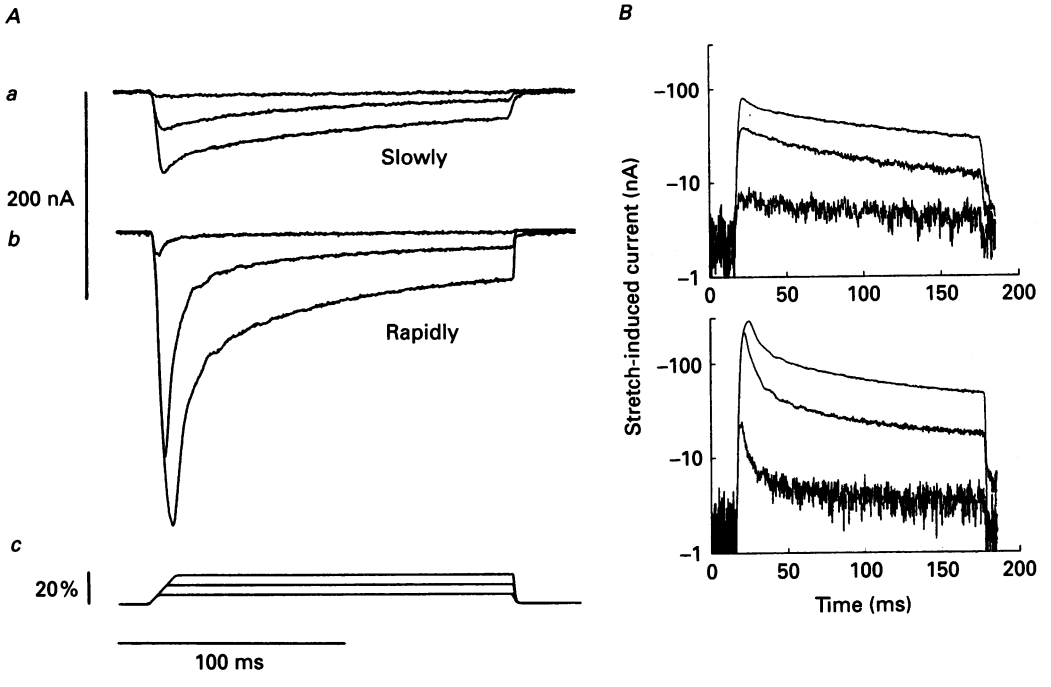


Fig. 5. Comparison of the decay phase of the receptor current between a slowly and a rapidly adapting SRN. *A*, receptor current of a slowly adapting SRN (*a*) and a rapidly adapting SRN (*b*) in response to three extensions (6, 12, 18%; *c*). *B*, the same data as in *A* plotted in a lin–log diagram. The decay phase has several time constants. The initial, fast decay was 19 ms in the slowly adapting SRN (mean from the 3 extensions) and 7 ms in the rapidly adapting SRN. The time constant (i.e. the slope of the graph) between 50 ms and end of the stretch seems to differ less between the two neurones.

allow comparison of data from the present study with data from previous studies using different stretch rates and to quantitate the relation between response and slope of the ramp we applied an extension amplitude of 20% with different slopes of the ramp. The steepest slope attainable in our set-up had a rise time of 2–3 ms, which corresponds to $10^4$% $s^{-1}$. Figure 6*A* shows that as the rise time increased the peak amplitude of the receptor current decreased (Fig. 6*Ac*). This was also found when the receptor potential was recorded (Fig. 6*Ab*). The difference between the receptor current and the receptor potential was that the current seemed to increase more at steeper ramps than the potential response. The peak amplitudes of the potential and current responses were fitted to a hyperbolic function:

$$y = a/(1+b/s), \quad (1)$$

where $a$ is the maximal peak potential or current amplitude in millivolts and nanoamps respectively and $b$ (% $s^{-1}$) is the ramp slope which gives the half-maximal activation (Fig. 6*B* and *C*). The smooth curves in Fig. 6*B* and *C* were drawn according to eqn (1) using $a = 33$ mV and $b = 360\%\ s^{-1}$ for the peak potential

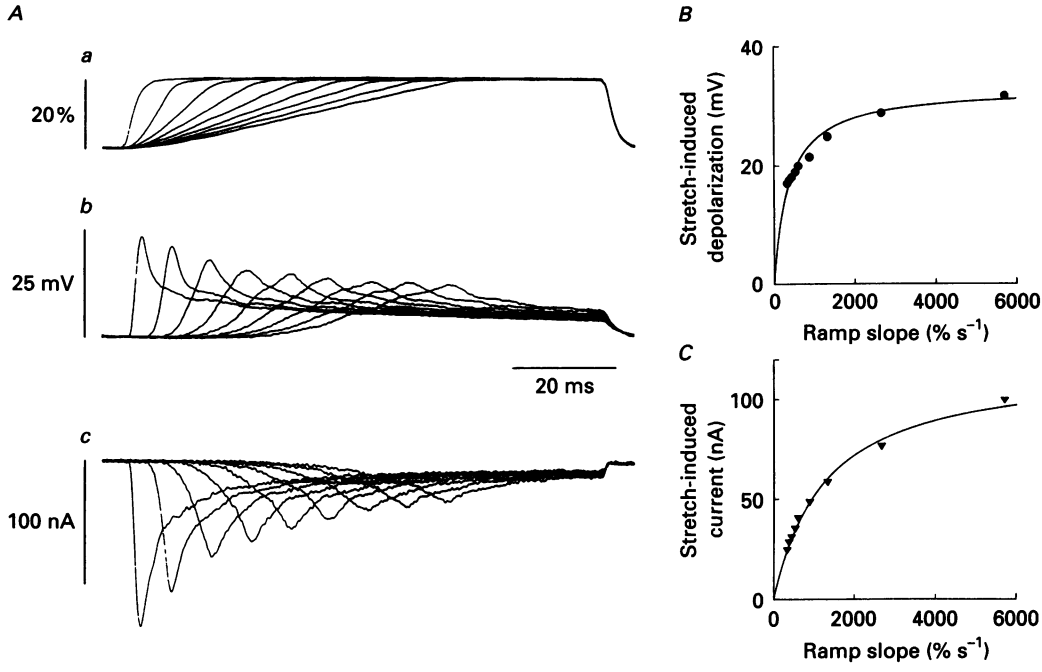


Fig. 6. Effect of slope of the ramp phase of the extension on the receptor potential and receptor current in the presence of 0·3 $\mu$M TTX. The same extension amplitude of 20% was applied to the receptor muscle with increasing slope of the ramp. *A a*, the extensions applied; *b*, superimposed receptor potential recordings; *c*, superimposed receptor currents recorded in voltage clamp, holding potential −65 mV. *B*, the peak amplitudes of the receptor potential were plotted against the slope of the ramp and the smooth curve was drawn according to eqn (1) ($y = a(1+b/s)$, where $a = 33$ mV and $b = 360\%\ s^{-1}$). *C*, the peak amplitude of the receptor currents was plotted against the slope of the ramp and the smooth curve was drawn according to eqn (1), where $a = 119$ nA and $b = 1286\%\ s^{-1}$.

response, and $a = 119$ nA and $b = 1286\%\ s^{-1}$ for the peak current response. It was found that the best fit was obtained using the current response which probably reflects the complex nature of the potential response (see above).

*Current–voltage relation of the stretch-induced current (SIC)*

Extending the receptor muscle with the cell clamped at a given potential level elicited a current (SIC) which was superimposed on the passive membrane current ($I_p$) component which is mainly due to the outward potassium currents associated with the change of the membrane potential. Since the total membrane current ($I_t$) is equal to the sum of the $I_p$ and SIC, subtraction of $I_p$ from $I_t$ gives the SIC alone. Brown *et al.* (1978) observed that more reliable *I–V* curves were obtained if the

neurone was clamped at +40 to +50 mV and the outward current was allowed to inactivate for some time. We followed a similar procedure: the neurone was initially clamped at +40 mV and the resulting outward current was allowed to inactivate for 20–30 s before the first extension was applied. The potential was then changed in

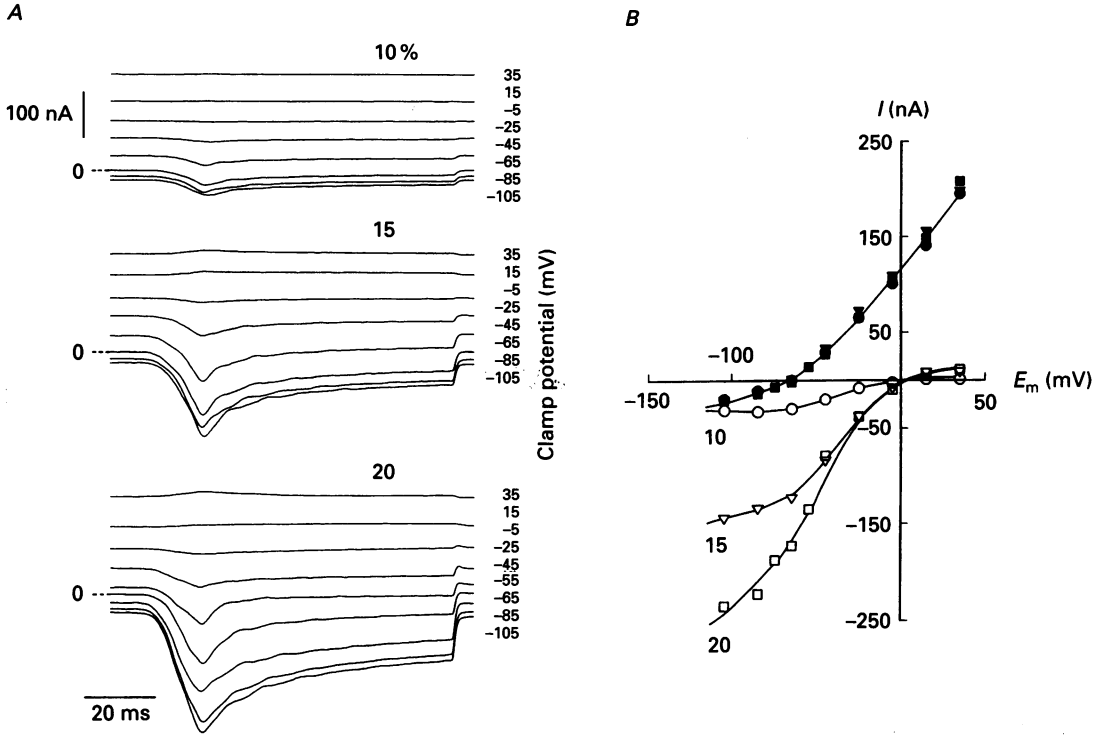


Fig. 7. Current–voltage relations of stretch-induced currents at three different extension levels in the presence of 0·3 μM TTX. The extension was applied about 20 s after the cell had been voltage clamped to the indicated level. *A*, current responses in a rapidly adapting neurone obtained using 10, 15, 20% of extension at the indicated potential levels. *B*, the current–voltage relations for the peak amplitudes of the stretch-induced current (open symbols) and for the pre-stretch current levels (filled symbols).

negative steps of 20 mV and stretches applied every 20 s. This was repeated 8–10 times so that the voltage range down to about −100 mV was covered. In Fig. 7 the *I–V* relation of the SIC together with the passive current component is shown for three different amplitudes of extension. The amplitude of the SIC was dependent on holding potential level as well as on the applied extension amplitude. The amplitude of the SIC was larger at negative holding potentials and at more positive holding potentials, the amplitude of the SIC decreased gradually and reversed sign at a certain potential level. As shown in Fig. 7 the reversal potential of the SIC was +5 mV in this cell and independent of the amplitude of the extension applied. In thirty-two cells studied the reversal potential ranged from 0 to +35 mV and the mean value was 16·2 ± 1·8 mV (mean ± S.E.M.).

From the mean reversal potential, the permeability ratio of $Na^+$ to $K^+$ was

calculated using the Goldman equation (Goldman, 1943). If it is assumed that $Ca^{2+}$ does not contribute to the SIC then $P_{Na}/P_K = 1{\cdot}51$. Taking into consideration the permeability for $Ca^{2+}$ estimated in separate experiments ($P_{Ca}/P_{Na} = 0{\cdot}44$, see below) and ignoring the contribution from $Mg^{2+}$, $P_{Na}/P_K$ was calculated to be 1·47.

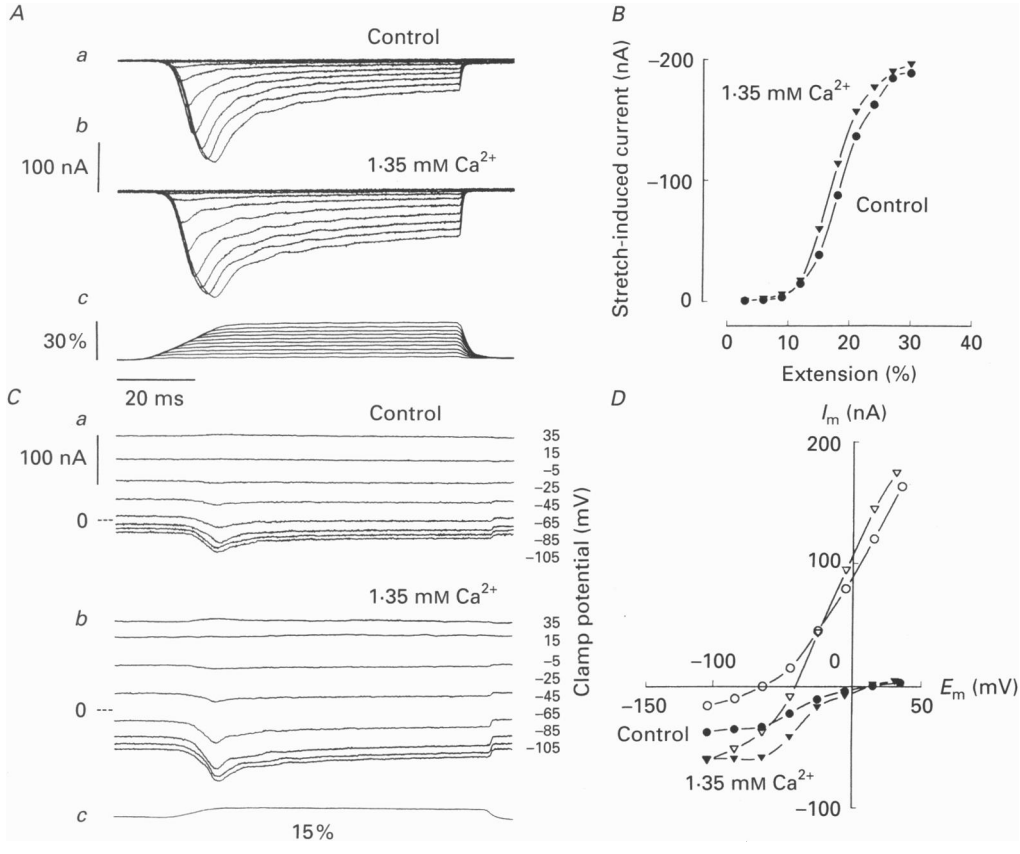


Fig. 8. Effects of lowering the extracellular calcium concentration on the transducer properties of a rapidly adapting stretch receptor neurone. *A*, current responses in control solution (*a*) and in low calcium solution (*b*) obtained by extension of the receptor muscle (3–30%, 1500% $s^{-1}$) as shown in *c*. *B*, plot of peak receptor current amplitude *versus* extension from recordings shown in *A*. *C*, the same cell as in *A* was voltage clamped at the indicated potential levels and a 15% extension was applied to the receptor muscle. *Ca*, current recordings in control solution; *Cb*, current recordings in low $Ca^{2+}$ solution; *Cc*, extension (1500% $s^{-1}$). *D*, the current–voltage relation obtained from the recordings in *C*. Filled symbols represent the peak stretch-induced current and open symbols represent pre-stretch current levels. Circles, control solution; triangles, low calcium (1·35 mM) solution.

*Effects of external divalent cation concentration on the transducer properties of the receptor membrane*

Divalent cations have been shown to move through the transducer permeability system (Edwards *et al.* 1981) and external $Ca^{2+}$ alters the receptor response in the slowly adapting neurone. It was shown by Brown *et al.* (1978) that decreasing the

external $Ca^{2+}$ concentration increased the amplitude of the peak SIC without any change in reversal potential and that the adaptive behaviour of the receptor response was changed. In order to see if this applies also to the rapidly adapting SRN the stimulus–response relation and the voltage dependence of the receptor current were investigated in the presence of different extracellular $Ca^{2+}$ concentrations.

TABLE 1. Composition of solutions (mM)

| | NaCl | KCl | $CaCl_2$ | $MgCl_2$ | Hepes | TEA | 4-AP |
|---|---|---|---|---|---|---|---|
| Control solution (NAS) | 200 | 5·4 | 13·5 | 2·6 | 10 | — | — |
| Isotonic calcium solution | — | 5·4 | 160 | — | 10 | — | — |
| Low calcium (1·35 mM) solution | 200 | 5·4 | 1·35 | 14·7 | 10 | — | — |
| Isotonic magnesium solution | — | 5·4 | 13·5 | 160 | 10 | — | — |
| TEA + 4-AP-containing solution | 150 | 5·4 | 13·5 | 2·6 | 10 | 50 | 5 |

The pH of the solutions was adjusted to 7·4 by titrating appropriate salts of NaOH or KOH. All solutions were composed to give an osmolarity of 420 mosmol$^{-1}$.

Lowering the external $Ca^{2+}$ concentration to 10% of the standard value slightly increased the amplitude of the SIC (Fig. 8*A*). As can be seen in Fig. 8*B* the effect was most apparent between 15–20% stretch. Also, the steady-state amplitude of the SIC seemed to increase more than the peak amplitude (Fig. 8*A*). The reduction of the extracellular $Ca^{2+}$ concentration increased the slope of both the passive and SIC *I–V* relations indicating a general conductance increase of the membrane. No change in reversal potential was observed. Figure 8*C* shows the current responses and the *I–V* relation (*D*) for an extension amplitude of 15%.

To study the permeability to divalent ions of the stretch-activated permeability system, cells were exposed to isotonic solutions of $CaCl_2$ and $MgCl_2$ (see Table 1). The isotonic $Ca^{2+}$ solution caused a hyperpolarization of 5–10 mV. The cell tolerated this solution for up to 10 min whereas longer exposure caused a slight depolarization. As shown in Fig. 9*A* the receptor current amplitude decreased in isotonic $Ca^{2+}$ with the reduction being dependent on the amplitude of extension (Fig. 9*B*). The maximum reduction (75%) of the peak current was found at an extension of between 12 and 15%, whereas the reduction at 30% extension was only 36%. It was also seen that the reduction in current at the end of the stretch was larger than at the peak of the current response. This indicates that both the permeability and the kinetic properties of the transducer permeability system are affected by $Ca^{2+}$. The permeability ratio of $Ca^{2+}$ to $Na^+$ was determined using the equation derived by Edwards, Ottoson, Rydqvist & Swerup (1981):

$$\frac{P_{Ca}}{P_{Na}} = \frac{1}{4}\frac{E_m}{E'_m}\frac{[Na^+]_o\{1-\exp(2FE'_m/RT)\}\,SIC_{Ca}}{[Ca^{2+}]'_o\{1-\exp(FE_m/RT)\}\,SIC_{Na}}, \quad (2)$$

where $'$ denotes the parameters in high $Ca^{2+}$ solution, $E_m$ is the membrane potential at which SIC was recorded and $[Na^+]_o$ and $[Ca^{2+}]'_o$ are ion activities. For $[Na^+]_o$ 200 mM and an activity coefficient of 0·74 was used and for $[Ca^{2+}]'_o$ 160 mM and 0·24 was assumed (Edwards *et al.* 1981). $F$, $R$ and $T$ have their usual meanings. Because the reduction in SIC was not linearly related to extension (Fig. 9*B*) we chose

the mean SICs at high extension where we assume that the open probability for stretch-activated channels is close to 1.

Inserting the appropriate values into eqn (2) a value of $P_{Ca}/P_{Na} = 0{\cdot}44 \pm 0{\cdot}06$ (mean $\pm$ S.E.M., $n = 4$) was obtained, which is in good agreement with what was found for the slowly adapting receptor (0·3, Edwards *et al.* 1981).

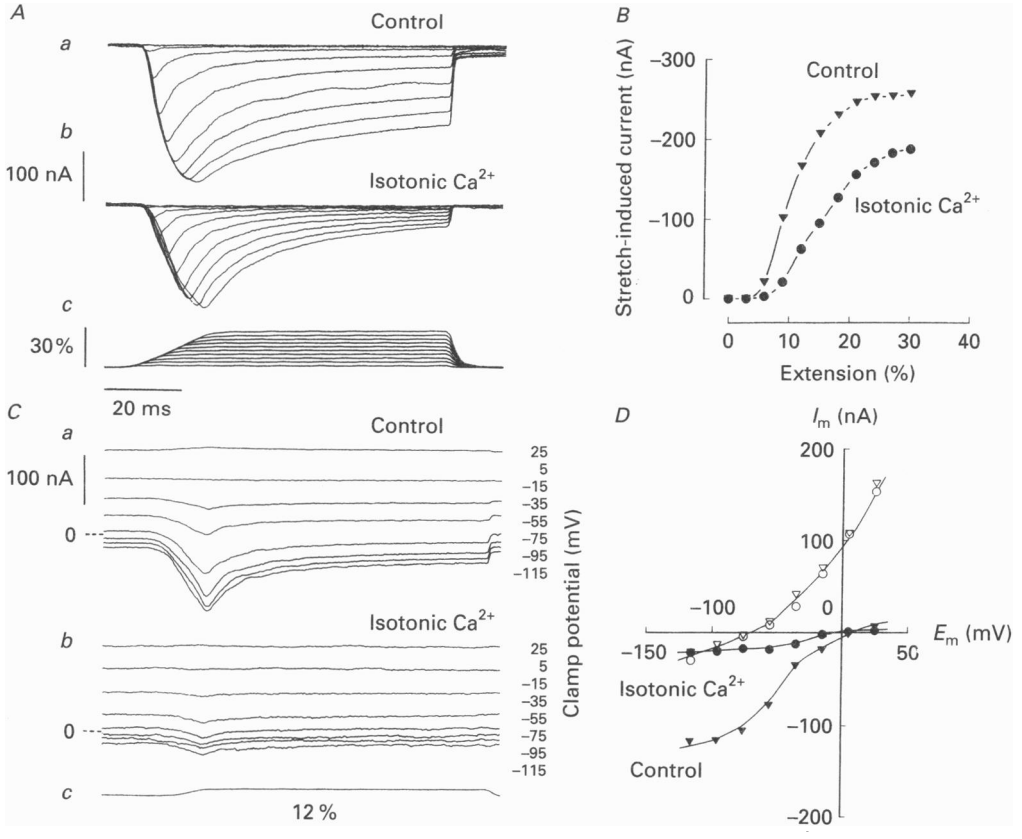


Fig. 9. Effects of isotonic calcium solution on the transducer properties of a rapidly adapting stretch receptor neurone. *A*, current responses in control solution (*a*) and in isotonic calcium solution (*b*) obtained by extension of the receptor muscle (3–30%) as shown in *Ac*. *B*, plot of peak receptor current amplitude *vs.* extension from recordings shown in *A*. *C*, the same cell as in *A* was voltage clamped at indicted potential levels and a 12% extension was applied to the receptor muscle. *Ca*, current recordings in control solution; *b*, current recordings in isotonic $Ca^{2+}$ solution; *c*, extension (12%, 1500% $s^{-1}$). *D*, the current–voltage relations obtained from the recordings in *C*. Filled symbols represent the peak stretch-induced current and open symbols represent pre-stretch current levels. Circles, control solution; triangles, isotonic calcium solution.

The effect of isotonic $Ca^{2+}$ solution on SIC (12% extension) at different clamp potentials is shown in Fig. 9*C* and the corresponding *I–V* relations are illustrated in Fig. 9*D*. There was a considerable reduction of the SIC over the entire voltage range in isotonic $Ca^{2+}$ solution, which corresponds to a decrease in conductance of the transducer permeability system; however, only a small shift of the reversal potential was observed.

With high $Mg^{2+}$ solutions it was found necessary to add $Ca^{2+}$ since application of solutions with $Mg^{2+}$ as the only cation severely depolarized the neurones (cf. Table 1). Cells were observed to tolerate this $Ca^{2+}$–$Mg^{2+}$ solution for limited periods of time whereas longer exposure caused depolarization. Similar to the effect of isotonic $Ca^{2+}$

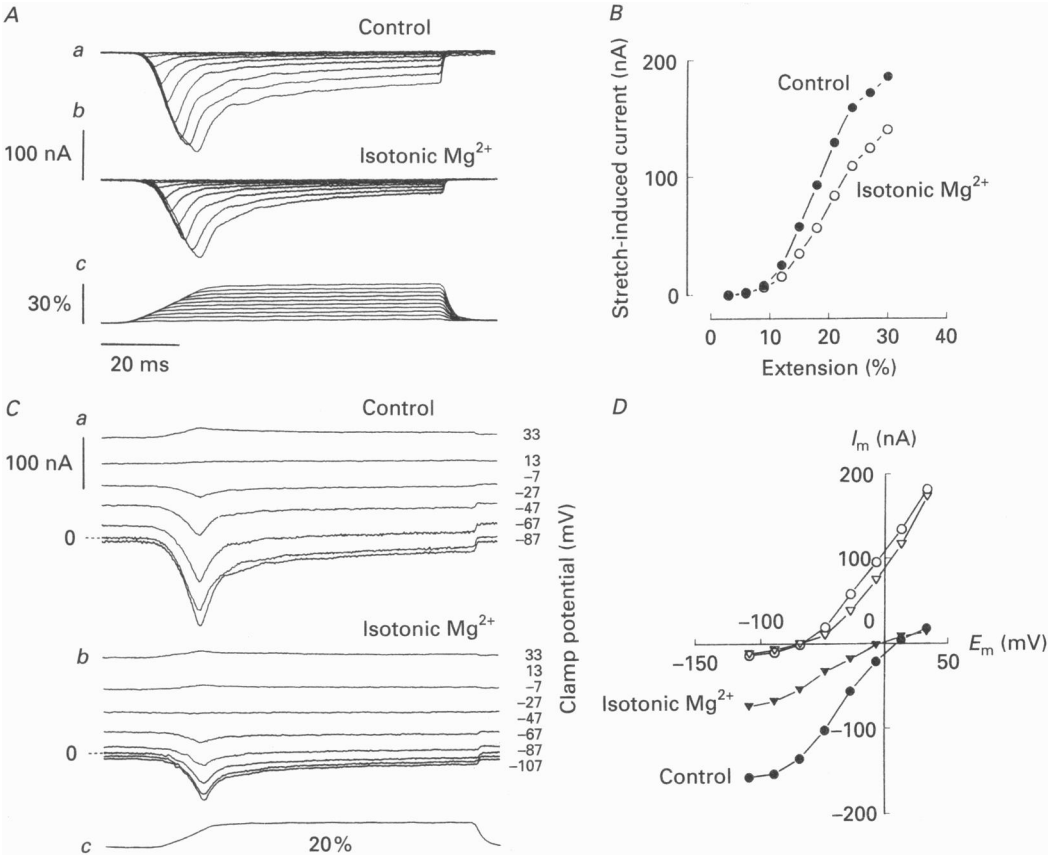


**Fig. 10. Effects of isotonic magnesium solution on the transducer properties of a rapidly adapting stretch receptor neurone. *A*, current responses in control solution (*a*) and in isotonic magnesium solution (*b*) obtained by extension of the receptor muscle (3–30%, 1500% $s^{-1}$) as shown in *c*. *B*, peak current response *versus* extension from data shown in *A*. *C*, the same cell as in *A* was voltage clamped at the indicated potentials and the receptor muscle was subjected to a 20% extension. *Ca*, the receptor currents in control solution; *b*, receptor currents in isotonic magnesium solution; *c*, extension (20%, 1500% $s^{-1}$). *D*, the current–voltage relation obtained from the recordings in *C*. Filled symbols represent the peak stretch-induced current and open symbols represent pre-stretch steady-state level of the outward currents. Circles, control solution; triangles, isotonic magnesium solution.**

solutions, high $Mg^{2+}$ concentration was observed to reduce the amplitude of SIC at all stretch amplitudes (Fig. 10*A* and *B*). The permeability ratio of $Mg^{2+}$ to $Na^{+}$ (substituting $Mg^{2+}$ for $Ca^{2+}$ in eqn (2)) obtained from four cells using the SICs at large extensions was $0{\cdot}60 \pm 0{\cdot}05$ (mean ± S.E.M., 4 cells). The same $Mg^{2+}$ activity coefficient as for calcium and other appropriate values were used.

The voltage dependence of the reduction of SIC in isotonic $Mg^{2+}$ solution was similar to that observed in isotonic $Ca^{2+}$ solution although the absolute reduction was smaller in the high $Mg^{2+}$ solution (Fig. 10*C* and *D*). Contrary to that observed at high extracellular $Ca^{2+}$ there was a shift in the reversal potential of $16 \pm 1{\cdot}2$ mV towards more negative potentials (mean $\pm$ S.E.M., 4 cells).

## DISCUSSION

Of the large number of studies on the transducer properties of crustacean stretch receptors few have been devoted to the rapidly adapting stretch receptor. Nakajima and Onodera (1969*a*, *b*) concluded that the adaptive difference between the slowly and rapidly adapting stretch receptors could not be explained by the viscoelastic properties of the receptor muscle of the primary transducer properties of the receptors but had to be the result of differences in the so-called encoder properties.

In this study we found that the impulse firing of the rapidly adapting SRN in response to a maintained stimulus, in most cells only lasts for less than 1 s. However, in a few cells prolonged impulse firing was seen (Fig. 1*C* and *D*) which indicates that there is a considerable variation.

The similarity in impulse firing with electrical and mechanical stimulation as shown in Fig. 1 supports the notion that the impulse response is, at least partly, independent of mechanical factors and/or the transducer permeability properties, i.e. stretch-activated (SA) channels (cf. Nakajima & Onodera 1969*a*, *b*). However, in contrast to the difference in impulse adaptation between the slowly and rapidly adapting SRN using long lasting extensions there is no obvious difference in adaptive behaviour in the 100 ms range as seen in Fig. 2*A* (cf. Swerup, 1983; Rydqvist & Swerup, 1991) although an accurate comparison between the two neurones is difficult to make. This is also true for the receptor potential. On the other hand, a careful analysis of the receptor current (stretch-induced current, SIC) as shown in Fig. 5 reveals that the decay phase (or adaptation) of the transient response is more rapid in the rapidly adapting SRN than in the slowly adapting SRN (receptors from the same abdominal segment). The mean time constant for the fast initial falling phase in the slowly adapting is about three times that of the rapidly adapting SRN. This property unquestionably contributes to adaptation but it should be kept in mind that this is less important for late adaptation taking place in the seconds range.

The stimulus–response relations were studied with regard to impulse frequency, receptor potential and receptor current (or SIC). The maximum slope of the relation between extension and impulse response (log–log plot) from eight cells was 2·9. This cannot be compared to the slowly adapting SRN because of lack of data. The log–log relation between extension and peak receptor current was sigmoidal and the mean maximum slope for twenty-eight cells was $4{\cdot}7 \pm 0{\cdot}25$ (Fig. 4*D*). This is larger than that found in the slowly adapting neurone ($3{\cdot}0 \pm 0{\cdot}13$, Rydqvist & Swerup, 1991). At small extensions ($< 5\%$) the slope seems to be close to one which was also found in the slowly adapting SRN (Johansson & Rydqvist, 1983; Rydqvist & Swerup, 1991). At large amplitude extensions ($> 25\%$) the slope decreases again, reflecting the fact that the SA-channels approach an open probability of one (cf. Erxleben, 1989), i.e. saturation of the stretch-activated permeability system. The difference in the

stimulus–response relation between the slowly and rapidly adapting receptor could be due either to receptor muscle properties or SA-channel differences. Nakajima & Onodera (1969*b*) concluded from tension measurements that the viscoelastic properties do not play a major role in determining the transducer properties. However, recent studies (B. Rydqvist & N. Purali, unpublished observations) indicate that differences in viscoelastic properties between the two types of receptor muscles may contribute to the difference in the stimulus–response properties. Since no patch clamp data on SA-channels in the rapidly adapting SRN are available it is not possible to draw definite conclusions. However, the steeper slope of the stimulus–response relation of the rapidly adapting SRN is consistent with the more rapid decay of the receptor current and the potential response, and an earlier termination of impulse firing.

The mean maximum current at 30% extension (where probability of SA-channel opening is close to 1) was found to be $190 \pm 44$ nA (mean $\pm$ S.E.M., 28 cells) in the rapidly adapting SRN as compared to $107 \pm 10$ nA (mean $\pm$ S.E.M., 10 cells) in the slowly adapting SRN (Rydqvist & Swerup, 1991) indicating that the total number of SA-channels is larger in the rapidly adapting SRN. Since the neurones seem to be of equal size (Rydqvist & Purali, 1991) either the density of SA-channels is higher in the dendritic area or the dendritic area is larger relative to the total size of the neurone. However, it should be kept in mind that there is a considerable variation in receptor current amplitude between different preparations (cf. Fig. 4*C* and Fig. 5).

*Permeability properties of the stretch-activated permeability system*

The properties of the stretch-activated permeability system in the rapidly adapting SRN were analysed by measuring the stretch-induced current at different potential levels and determining the reversal potential for the SIC. A mean value of $+16 \pm 1{\cdot}8$ mV (mean $\pm$ S.E.M., 32 cells) was obtained which is very close to the value found in the slowly adapting SRN: $13 \pm 6{\cdot}5$ mV (mean $\pm$ S.D., 26 cells). If we assume, as for the slowly adapting neurone (Brown *et al.* 1978), that $Ca^{2+}$ does not contribute to the stretch-induced current (SIC) then $P_{Na}/P_K = 1{\cdot}51$, which is somewhat lower than for the slowly adapting SRN ($P_{Na}/P_K = 1{\cdot}6$).

The present study shows that the stretch-activated permeability system (SA-channels) in the rapidly adapting neurone is permeable to both $Ca^{2+}$ ($P_{Ca}/P_{Na} = 0{\cdot}44$) and $Mg^{2+}$ ($P_{Mg}/P_{Na} = 0{\cdot}60$) ions, similar to the finding in the slowly adapting neurone (0·3 and 0·4 respectively). Thus, the permeability ratio for different ions, $P_{Na}:P_K:P_{Ca}:P_{Mg}$, is $1:0{\cdot}68:0{\cdot}44:0{\cdot}60$.

The stimulus–response relation in high $Ca^{2+}$ as shown in Fig. 9*B* demonstrates that the effect of $Ca^{2+}$ ions depends on the degree of extension. This suggests that the probability of opening of the SA-channels is also affected. As seen in Fig. 10*B* this is not the case in high $Mg^{2+}$ solutions where the reduction of SIC seems to be extension independent.

An alternative explanation for the reduction of SIC in high divalent cation concentrations could be differences in viscoelastic properties of the receptor muscle in control and high divalent cation solutions. In fact, preliminary experiments indicate that the tension response in the receptor muscle is decreased in a high $Mg^{2+}$ solution whereas it is not affected in high $Ca^{2+}$ solution.

In summary, it is confirmed that using ramp-and-hold extensions, impulse firing

seldom exceeds 1 s, compared with the prolonged firing seen in the slowly adapting SRN. To some extent the rapid adaptation of the impulse firing can be explained by differences in the permeability properties of the transducer system: the decay phase or adaptation of the receptor current is faster in the rapidly adapting than in the slowly adapting SRN and the stimulus–response relation of the SIC is steeper than that found in the slowly adapting SRN. Whether this is due to differences in viscoelastic properties of the receptor muscles or to differences in SA-channel properties has to be studied further. In addition, other factors like potassium permeability systems can contribute to impulse adaptation (Rydqvist & Zhou, 1989; Rydqvist & Purali, 1991). However, to explain fully the difference in adaptive behaviour other factors like the $Na^+$ transport system(s) must also be taken into account.

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