Action Potential and Sodium Current in the Slowly and Rapidly Adapting Stretch Receptor Neurons of the Crayfish (Astacus astacus)

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Purali, Nuhan and Bo Rydqvist. Action potential and sodium current in the slowly and rapidly adapting stretch receptor neurons of the crayfish (Astacus astacus). J. Neurophysiol. 80: 2121–2132, 1998. Action potentials (APs) and sodium current from the slowly and the rapidly adapting stretch receptor neurons in the crayfish (Astacus astacus) were recorded with a two microelectrode voltage- and current-clamp technique. In the rapidly adapting neuron the APs had a duration of 3.2 ± 0.2 ms (means ± SE) and an amplitude of 55.2 ± 1.5 mV. In the slowly adapting receptor neuron APs had a duration of 4.1 ± 0.2 ms and an amplitude of 79.9 ± 2.0 mV. APs in the rapidly adapting neuron had a larger amplitude if they were recorded from the axon. In the rapidly adapting neuron adaptation of the impulse response was prolonged by hyperpolarization or by exposure to scorpion venom. Also, sinusoidal current stimulation added to the current steps prevented impulse adaptation. Block of the potassium currents in the slowly adapting neuron resulted in a rapid adaptation of the impulse response. The maximum sodium current amplitude was 313 ± 15 nA in slowly adapting neuron and 267 ± 11 nA in the rapidly adapting neuron. The current-voltage relationship showed a hump most marked in the slowly adapting neuron and abolished when a depolarizing prepulse was given. In the rapidly adapting neuron the inactivation starts at a more negative potential (E_i = −45 mV) and is faster compared with the slowly adapting neuron (E_i = −41 mV). The crude scorpion venom of Leiurus quinquestriatus (ScVLq) shifted h_0 curve toward more positive potentials and slowed down the rate of inactivation. The results indicate the possible presence of more than one Na^+ channel population and that the relative density and the spatial distribution is different in the slowly and rapidly adapting neuron. The difference contributes to the adaptive properties of the two receptor neurons.

INTRODUCTION

The stretch receptor organ of the crustaceans, which was first described by Alexandrowicz (1951), was since the subject of a number of studies regarding its function (for a review see Swerup and Rydqvist 1992). A substantial amount of work was done to characterize the transducer mechanisms of the slowly and rapidly adapting receptor regarding its viscoelastic properties (Nakajima and Onodera 1969b; Rydqvist et al. 1990, 1994), its transducer properties in both crayfish and lobster (Brown et al. 1978; Erxleben 1989; Rydqvist and Purali 1993; see also Swerup and Rydqvist 1992), and the voltage-gated ion currents present in the neuron (Edman et al. 1986, 1987a,b; Purali and Rydqvist 1992; Rydqvist and Purali 1991; Rydqvist and Zhou 1989). The outward potassium current was studied in the rapidly adapting neuron (Rydqvist and Purali 1991), and it was found that the kinetics of this permeability system differed in several ways compared with that in the slowly adapting neuron (Rydqvist and Zhou 1989). Furthermore, by using tetraethylammonium chloride (TEA) and 4-aminopyridine (4-AP), Purali and Rydqvist (1992) found evidence that the outward rectifying and inactivating outward current may be the result of two potassium channel populations and that the relative density of these two channels differs in the slowly and rapidly adapting receptors. However, the difference in impulse response adaptation cannot be fully explained by the viscoelastic properties of the receptor muscles, by the differences in the K^+ currents, and by stretch-activated current of the receptor neurons. Our hypothesis was that part of the adaptation is due to differences in the properties of the Na^+ channels or the spatial distribution of the Na^+ channels. This is suggested by the findings that the adaptive properties of the two neurons are similar independent of whether impulses are initiated by stretch or electrical current injected into the neuron (Nakajima and Onodera 1969a; Rydqvist and Purali 1993). Although the action potentials (APs) were studied in the lobster (Grampp 1966a,b,c) the fast sodium current generating the APs in the crayfish was not investigated comparatively in rapidly and the slowly adapting neurons (see however Loewenstein et al. 1963; Swerup and Rydqvist 1985). To see if the AP generating mechanism is different in the slowly and rapidly adapting stretch receptor neuron, we studied the APs and the voltage-gated sodium current, known to be responsible for the APs (Loewenstein et al. 1963), in the two neurons.

METHODS

General procedures

The experiments were carried out on the slowly and rapidly adapting stretch receptors of the crayfish Astacus astacus. In the use of the experimental animals, the national guidelines were followed, and ethics committee approval was obtained. The receptor neuron together with its muscle was isolated and mounted in a small chamber that could be perfused at a rate of ≤4 ml/min. The experiments were carried out at 10°C to facilitate measurements of the fast sodium current. The temperature was controlled by a pair of Peltier elements driven by a feedback amplifier system attached to a temperature sensor close to the preparation (Moser et al. 1979). The composition of the normal astacus saline was (in mM) 207 NaCl, 5.4 KCl, 13.5 CaCl_2, and 2.6 MgCl_2 (van Harreveld 1936), buffered to pH 7.4 with 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid. In some experiments 1 mM 4-AP
and 10 mM TEA were used to block the potassium currents, and
tetrodotoxin (TTX; 0.3 μM) was routinely used to block the sodi-
um current. The crude scorpion venom of Leiurus quinquestriatus
(ScVLq) from North Africa was used at a concentration of 10⁻²
mg/ml (V-5251, Lot 61H0307, Sigma Chemical, St. Louis, MO).
To improve voltage-clamp conditions the axon was pinched 175–
300 μm from the cell soma in the slowly adapting neuron. In the
rapidly adapting cell the axon was pinched 1–2 mm from the soma
because closer pinching abolished the inward sodium current.

Stimulation and recording

The apparatus and the methods for stimulation and recording
were described previously (Brown et al. 1978; Johansson and
Rydqvist 1983; Rydqvist and Purali 1993). In these experiments
the membrane potential and current were recorded with two micro-
electrodes in current-clamp or voltage-clamp modes with an Axo-
clamp 2B (Axon Instrument, Foster City, CA) two-microelectrode
voltage-clamp amplifier. The signals were filtered at 6,000 Hz.
The potential and current signals were sampled by a computer-aided
stimulation and sampling equipment (Digidata/pClamp 6, Axon
Instrument) and stored in a computer. The sampling frequency was
set to 25 kHz or in some cases to 40 kHz. Analysis of the recorded
data were done with pClamp 6 programs.

The micropipettes (GC150F, Clarke Electromedical
Instruments, Reading, UK) for membrane potential measurements and
current injection were filled with 3 M KCl (2–5 MΩ). The refer-
celectrode consisted of a micropipette filled with 3 M KCl-
Agar mixture connected to an Ag/AgCl pin.

The results are expressed as means ± SE. Comparison between
groups of data were made by Student’s t-test. A P value <0.05
was considered statistically significant.

RESULTS

The APs

Electrical stimulation, or extending the receptor muscle,
gave rise to APs in the rapidly and the slowly adapting
stretch receptor neurons. The waveform and the other
properties of the APs were similar independent of whether the
neurons were stimulated by current injection into the cell
soma or by extending the receptor muscle. Representative

![Fig. 1](image1)

**FIG. 1.** Comparison of the soma action potentials (APs) in a rapidly and slowly adapting receptor neuron. Potential
responses were elicited by stimulation through a second intracellular electrode in (A) a rapidly adapting neuron, maximum
stimulus 96 nA, and in (B) a slowly adapting neuron, maximum stimulus amplitude 14 nA. Stimulus duration is 0.5 ms,
and waveform is shown at the bottom. C: typical AP from a rapidly and slowly adapting neuron were superimposed (solid
lines), and the amplitude of the AP from the rapidly adapting neuron was normalized to give the same peak amplitude as
the latter (dashed lines).

![Fig. 2](image2)

**FIG. 2.** Comparison of the APs recorded in a rapidly adapting neuronal soma to those recorded in the
axon 250 μm from the somal electrode. Solid lines are the potential recordings from axon, and the dotted
lines are the potential recordings from the cell soma. A: stimulation in axon (3 current levels). B: stimulation
in the soma (3 current levels). C: part of the recordings displayed in B shown on an expanded timescale.
AP recordings from a rapidly and a slowly adapting neuron are shown in Fig. 1. The critical potential level for firing, the threshold, in either receptor neuron was similar to −55 mV. However, the amount of current needed to depolarize the cell to the threshold was 35% larger in the rapidly adapting receptor neurons.

The absolute amplitude of the APs was smaller in the rapidly adapting neurons (55.2 ± 1.5 mV, mean ± SE, n = 12) compared with that in the slowly adapting neurons (79.9 ± 2.0 mV, n = 11). Also, the afterhyperpolarizations following the APs in the slowly adapting neuron shown in Fig. 1B were prolonged ~15 ms compared with the rapidly adapting neuron. The APs from the slowly adapting neurons had a longer duration (4.1 ± 0.2 ms, n = 10) compared with the rapidly adapting neuron (3.2 ± 0.2 ms, n = 10). This difference is mainly due to the increased rate of repolarization in the rapidly adapting neuron.

In the rapidly adapting neuron the AP amplitude was larger in the axon than that recorded in the cell soma (Fig. 2). Furthermore, the axonal AP amplitude had a steeper rise than that recorded in the cell soma. The findings were the same irrespective of the site of stimulation (Fig. 2).

When longer stimulations were applied trains of actions potentials were evoked in both receptor neurons. However, an apparent difference in the rate of adaptation was seen between the two neurons. In the slowly adapting neuron, long depolarizing current injections at low amplitudes initiated a continuous impulse response. If the amplitude of the current stimulus was increased to a certain level (10–15 nA) the amplitude of the APs gradually decreased and the firing ceased (Fig. 3A, top recordings). The impulse responses were recovered if the saturating current stimulus was reduced (Fig. 3B). In contrast, in the rapidly adapting neuron (Fig. 3C), a reduction of the current amplitude did not restore impulse firing.

When mechanical stimulation was used (~40% extension), continuous firing was a characteristic response in the slowly adapting neuron (Fig. 3A, middle recordings). The amplitude of the individual APs gradually increased if mechanical stimulus was used whereas current injection caused a reduction of the AP amplitude with time (Fig. 3A). This is probably due to the rapidly adapting receptor current (bottom traces in Fig. 3A), underlying the impulse firing.

The firing duration in the rapidly adapting neuron was improved if the cell was hyperpolarized (Fig. 4A), and a continuous firing could be maintained if a sine wave (9 nA 50 Hz), was superimposed on a rectangular current stimulus (14 nA) or vice versa (Fig. 4B). The APs were paced by the frequency of the sinusoidal stimulation and were fired during the initial part of the rising phase of the sine wave (Fig. 4C). It is concluded that inactivation of the Na⁺ permeability system is important in determining the duration of the impulse train.

FIG. 3. Comparison of the firing behavior to various stimulations. A: impulse responses to long electrical (top traces) and mechanical (middle traces) stimulation together with associated receptor currents recorded in 0.3 μM tetrodotoxin (TTX, bottom traces) in a slowly adapting neuron. B: potential response to a change in the current stimulus in a slowly adapting neuron. C: potential responses in a rapidly adapting neuron for a simple depolarizing stimulus (top trace) and a decrease in current amplitude from 10 to 6 nA (bottom trace). Stimulus waveform is shown at the bottom.
Because $K^+$ currents are known to reprime the $Na^+$ permeability system for the next AP by hyperpolarizing the neuron, we partially blocked the $K^+$ system by exposing the neuron to 1 mM 4-AP and 10 mM TEA. A small increase in the firing duration was observed (Fig. 5A) in the rapidly adapting neuron, whereas in the slowly adapting neuron a few APs only followed by some irregular depolarizations could be evoked (Fig. 5B).

To investigate the effect of the inactivating properties of the $Na^+$ system we exposed the neurons to scVLq, which has been shown to prolong inactivation of the sodium current in frog myelinated nerve and muscle (Catterall 1979; Koppenhöfer and Schmidt 1968; Stritcharz and Wang 1986). In the crayfish stretch receptor neurons ScVLq ($10^{-2}$ mg/ml) caused some changes both in potential and current responses, and some effects were different in the rapidly adapting neuron from those obtained in the slowly adapting neuron. The ScVLq effect was time dependent and was never appreciably reversed by washing out the toxin with the control solution.

Exposing the sensory neurons to ScVLq resulted in a depolarization in both receptor neurons, proportional to concentration and the duration of the exposure. The duration and the amplitude of the APs and the afterhyperpolarizations following the APs were broadened in both receptor neurons. When exposed to ScVLq the APs had a distinct biphasic decay in the rapidly adapting neuron, and a broadening of the APs was characteristic for the slowly adapting neuron (Fig. 6A). When this particular rapidly adapting neuron was electrically stimulated (14 nA) in normal saline, a single AP was elicited (Fig. 6B, top trace). After exposure of ScVLq the neuron showed a prolonged firing (Fig. 6B, middle trace), which was abolished when TTX was applied (Fig. 6B, bottom trace). Hence, the rapidly adapting neuron was converted into a more slowly adapting cell by the ScVLq exposure. In the presence of ScVLq the slowly adapting neuron developed spontaneous rhythmic depolarizations at rest (Fig. 6C, top trace), which were not seen in the rapidly adapting neuron (cf. Fig. 6B). These responses were...
blocked by TTX (Fig. 6C, bottom trace). Contrary to what was observed in the rapidly adapting neuron, stimulation of the slowly adapting neuron (10 nA) showed no difference in adaptive behavior after exposure to ScVLq (Fig. 6C, middle trace).

**Inward current**

Voltage clamping the receptor neurons to different positive potential levels elicited membrane current composed of a fast and large capacitative component followed by the ionic currents (Fig. 7, top traces). The capacitative current attained the peak amplitude instantaneously with the onset of the voltage command and decayed to background current level in <0.5 ms. The amplitude and the direction of the capacitative current were dependent on the level of the potential step; however, the time course of the decay was not. The ionic currents, in response to depolarizing voltage steps, were composed of an inward and an outward component. The inward component could be blocked selectively by TTX (Fig. 7, middle traces) or by Na⁺ substitution (unpublished observation). The inward sodium currents were then isolated by subtracting the current responses obtained in the TTX solution from the current response obtained in control solution (Fig. 7, bottom traces). An alternative method in isolating the Na⁺ current could have been to block the potassium currents by 4-AP and TEA. However, these substances only give a partial block at concentrations, which does not change the extracellular ion concentration (Purali and Rydqvist 1992).

In the slowly adapting neuron the voltage steps evoked inward current somewhat different from that observed in the rapidly adapting neuron. The peak amplitudes of the inward current in this neuron were significantly larger (313 ± 15 nA, n = 27) and decayed on a slower timescale (see Fig. 7) compared with those in the rapidly adapting neuron (267 ± 11 nA, n = 19) for the same voltage steps. Furthermore, the shape of the current was complex, and in some voltage steps a biphasic current response could be observed (Fig. 7, right panel). The biphasic responses were only seen in the slowly adapting neuron. Neither pinching the axon nor compressing against it with another glass pipette eliminated either of the peaks observed in the recordings from the slowly adapting neurons studied. The current voltage relationships of the inward current were also different (Fig. 8). In the rapidly adapting neuron the inward current-voltage relationship was steep during onset, whereas in the slowly adapting neuron the activation of the inward current covered a larger voltage range.

The two components of the inward current in the slowly adapting neuron differed in their potential dependence of activation. If the test pulses were delivered after a depolarizing prepulse of 10–30 mV (Fig. 9A), taking the cell to about −40 mV, the low threshold component could be removed leaving the high-threshold component isolated. The depolarizing conditioning prepulses shifted the I-E plots of the peak inward current toward more positive potential range (Fig. 9B). The shift was most obvious in the potential range between −50 and 0 mV, whereas the plots merged gradually into a common curve for the positive potential range. If the peak sodium current amplitudes obtained after a depolarizing conditioning pulse (Fig. 9B, open circles) were subtracted from those obtained after a hyperpolarizing prepulse (filled circles), the resulting current amplitudes gave an I-E plot (open triangles) shifted to more negative potentials.

This I-E plot is similar to that obtained in the rapidly
adapting neuron (cf. Fig. 8). A similar experiment on the rapidly adapting neuron resulted in a high-threshold component with a considerably smaller amplitude (unpublished observations). The components of the sodium current were more apparent if the neurons were voltage clamped to voltage ramps (10 V/s, Fig. 9C).

The slow rate of change in the voltage ramp gave rise to two distinct inward current peaks with different threshold and waveform. The high-threshold component was apparently larger in the slowly adapting neuron than that obtained in the rapidly adapting neuron.

The inward currents from both receptor neurons were subjected to a voltage- and time-dependent inactivation process, leading to a fast decay of the inward current. Voltage dependence of the inactivation ($h_\infty$) of the inward current was analyzed by comparing the peak current amplitude to a test pulse delivered after various holding potentials (Fig. 10). The experimental data points for $h_\infty$ versus potential ($E$) were fitted to Eq. 1

$$ h_\infty = \frac{1}{1 + \exp((E - E_h)/a)} \quad (1) $$

where $E$ is the membrane potential, $E_h$ is the potential at which $h_\infty$ is 0.5, and $a$ is a constant. The half-inactivation potential level for the inward current in the rapidly adapting neuron was $-45$ mV (4 cells) and $a = 6.0$ mV, different (but not significantly) from those for the slowly adapting SRNs where $E_h$ was $-41$ mV (4 cells) and $a = 5.1$ mV. The time constant of inactivation of the sodium system ($\tau_h$) was analyzed in two ways. For potentials larger than $-40$ mV the time constant was estimated from the decay of the sodium current by fitting of a single exponential (Fig. 11B). For potentials around the resting membrane potential the method devised by Hodgkin and Huxley (1952) was used (Fig. 11A). The resulting values for ($\tau_h$) for the slowly and the rapidly adapting neurons (Fig. 11C) show that the inward current decayed more slowly in the slowly adapting neurons compared with the rapidly adapting neurons.

Suppression of inactivation by ScVLq ($10^{-2}$ mg/ml) changed the inward current. The threshold potential for the activation of the inward current was not affected in either neuron (Fig. 12, Aa and Ab), but the amplitude became larger (filled circles). As shown in Fig. 9B the $I-E$ curve

FIG. 6. Effects of crude scorpion venom of Leiurus quinquestriatus (ScVLq) on APs and firing behavior. A: AP in control (dashed lines) and ScVLq ($10^{-2}$ mg/ml) containing solutions (solid lines). B: potential responses in a rapidly adapting neuron to rectangular current injection (14 nA) in control (top trace), ScVLq containing solution (middle trace), and after TTX (0.3 $\mu$M, bottom trace). C: steady-state depolarizations induced by ScVLq at rest in a slowly adapting neuron (top trace), the potential responses to rectangular current injection (10 nA) in ScVLq containing solution (middle trace), and after addition of TTX (0.3 $\mu$M, bottom trace). Note the absence of current stimulation in top trace in C.
for the inward current of the slowly adapting neuron showed a distinct hump (Fig. 12Ab), which was not seen in the rapidly adapting neuron (Fig. 12Aa). This hump in the I-E plot was enhanced in the slowly adapting neuron (Fig. 12Ab), whereas the form of the I-E plot in the rapidly adapting neuron was unaffected (Fig. 12Aa). The effect of ScVLq was potential dependent; the effect was less apparent at positive potentials (Fig. 12). In both receptor neurons ScVLq shifted the steady state of inactivation ($h_s$) curve toward more positive potentials, and the slope became less steep than the control (Fig. 12, Ba and Bb). Inactivation of the inward current was slowed down giving rise to long-lasting and larger current amplitudes than those recorded in control saline as shown when the cells were voltage clamped with voltage ramps (Fig. 12, Ca and Cb). The biphasic behavior of the inward current was also enhanced in the slowly adapting neuron as shown in Fig. 12Cb, where the neuron is voltage clamped with a ramp command potential. No difference in the current responses was observed if ScVLq were added after the block of the sodium channels by TTX. Thus ScVLq had no effect on the the potassium, capacitative, or leak currents.

FIG. 7. Comparison of the membrane current in a rapidly and a slowly adapting neuron. Top traces: superimposed membrane current to the voltage steps shown at the bottom. Middle traces: current responses to the same voltage steps in the presence of TTX (0.3 μM). Bottom traces: TTX sensitive component of the membrane current isolated by subtracting the individual responses of the middle traces from the corresponding current responses shown in the top traces. Voltage-clamp waveform is the same in all experiments, and indicated amplitudes are in reference to the resting potential. $E_{rest} = -70$ and $-61$ mV in the rapidly and the slowly adapting neurons, respectively.

FIG. 8. Comparison of the current-voltage relationships of the inward currents from a rapidly and a slowly adapting receptor neuron. Peak inward current amplitude is plotted against the level of the corresponding voltage step.
**Fig. 9.** Effects of different conditioning prepulses on the sodium current induced by incremental (5 mV) voltage steps up to +35 mV in a slowly adapting neuron. **A,** top traces: superimposed inward current responses to various test pulses delivered after a hyperpolarizing step to −85 mV ($E_r = −55$ mV). **A,** bottom traces: sodium current evoked by the same test pulses delivered after a depolarizing conditioning pulse to −40 mV. **B:** $I-E$ plot of the peak inward current responses displayed in **A** against the level of the test potential. Filled and open circles represent the peak amplitudes of top and bottom records in **A,** respectively. Triangles represent the difference of the corresponding peak amplitudes. **C:** sodium currents to a voltage ramp (10 V/s) in rapidly and slowly adapting neurons. Stimulus waveform is shown at the top, and the potentials are in reference to the resting level, −59 and −66 mV in the slowly and the rapidly adapting neurons, respectively.

**DISCUSSION**

Despite the large number of papers concerned with the transducer properties of the crustacean stretch receptors, few studies focused on the properties of the AP (Eyzaguirre and Kuffler 1955; Grampp et al. 1966a,b,c; Nakajima and Onodera 1969a) and in particular the fast sodium current underlying these potentials. Most studies were concerned with model building to construct AP responses (Edman et al. 1987a; Gestrelius 1983), but few studies on the sodium current were reported (Swerup and Rydqvist 1985). The scarcity of investigations is due to the fact that current recordings in these medium-sized cells are hampered by the difficulty of injecting sufficiently large current into the cells. However, the use of large electrodes (2–3 MΩ filled with KCl) and the pinching of the axon close to the soma to minimize space-clamp problems together with computer-aided stimulation and sampling facilities made it possible to conveniently isolate the Na⁺ and K⁺ currents. Also, cooling down the preparation results in slower current responses, which improves voltage-clamp conditions and makes analysis easier. We emphasized a comparison of the fast inward sodium current in the slowly and rapidly adapting stretch receptor neurons.

The amplitude of the AP was smaller in the rapidly adapting compared with that in the slowly adapting neuron (Fig. 1), in agreement with an earlier study on *Orconectes* (Nakajima and Onodera 1969a). However, the difference observed in *A. astacus* (24 mV) was larger compared with that of *Orconectes* (8 mV). The difference could be explained by
the smaller Na⁺ current in the rapidly adapting neuron (267 nA) compared with that of the slowly adapting neuron (313 nA). Another possible reason is that the recordings are made through a microelectrode placed in the cell soma. The signal amplitude may be reduced if the AP is spread electrotonically from an AP initiation site located further away along the axon hillock in the rapidly adapting neuron compared with that in the slowly adapting neuron (Edwards and Otteson 1958). The hypothesis is supported by the finding that the axonal APs are somewhat larger and have a faster rate of rise than those recorded in the cell soma of the rapidly adapting neuron (Fig. 2). Furthermore, the capacity of the rapidly adapting neurons to generate AP is completely abolished if the axon is pinched close to the soma. However, the amplitude of the axonal APs from the rapidly adapting neuron is smaller than that recorded in the slowly adapting neuronal soma or axon. Hence the differences in the sodium current should be a more important factor determining the differences in the AP amplitude than the signal attenuation caused by the electrotonic spread. APs in the slowly adapting neuron have a longer duration than those in the rapidly adapting neuron. The rate of rise of APs is similar (Fig. 1C), but the decay is slower in the slowly adapting neuron, indicating some kinetic differences in the AP generating permeability changes. As expected, in the rapidly adapting neuron the inactivation of the sodium channels takes place at more negative potentials (Fig. 10) and at a faster rate compared with that in the slowly adapting neuron (Fig. 11). The difference in the AP waveforms may be due partly to the different kinetic properties of the potassium currents as analyzed in previous studies (Rydqvist and Purali 1991; Rydqvist and Zhou 1989). The faster rate of K⁺ current activation, taking off at more negative potentials, may terminate the APs more quickly in the rapidly adapting neuron than that in the slowly adapting neuron. The longer lasting afterhyperpolarizations in the slowly adapting neuron may well be due to differences in the ratio of two potassium channel populations (Purali and Rydqvist 1992).

Long stimulation evokes trains of APs, with different properties in the slowly and the rapidly adapting neurons. In the slowly adapting neuron the rapid decay of the receptor current is the major cause of nonadapting impulse responses even at huge levels of extensions (Fig. 3A). The gradual increase in AP amplitude to long-lasting mechanical stimulation may well stem from the decay in the receptor current (Fig. 3A). The increase in the membrane conductance, caused by opening of the stretch-activated channels, is another factor preventing impulse adaptation when mechanical stimulation is used. The adaptation of the impulse responses to large rectangular current injections is probably due to a depolarization-induced transition of the sodium channels into an inactivated state (cf. Fig. 10). The same factor may be the reason for the termination of the impulse responses when potassium currents are blocked by TEA and 4-AP. Under physiological conditions the receptor potential amplitude is limited to a certain potential level so that complete inactivation of the sodium channels is avoided (Rydqvist and Purali 1993). If this limitation is eliminated by either large current injections or potassium channel blockade, adaptation can be observed even in the slowly adapting receptor
FIG. 12. Effects of ScVLq (10^{-2} mg/ml) on the sodium current in the receptor neurons. $A_a$ and $A_b$: $I-E$ plots of the peak sodium current to voltage steps in control (open circles) and ScVLq containing solution (filled circles) in rapidly (top) and slowly adapting receptor neurons (bottom). $B_a$ and $B_b$: steady state of inactivation obtained in the control (hollow circles) and ScVLq containing solution (filled circles) in rapidly (top) and slowly adapting receptor neurons (bottom). $C_a$ and $C_b$: membrane current to a voltage ramp (9.17 V/s) in rapidly (top) and slowly adapting (bottom) neurons obtained in control solution (solid lines), ScVLq containing solution (dashed lines), and after addition of TTX (0.3 μM, dotted lines). The stimulus waveform is shown at the top, and the potentials are in reference to the resting level, −61 and −68 mV, in the slowly and the rapidly adapting neurons, respectively.

The idea is further supported by the finding that firing could be recovered in the slowly adapting neuron by reducing the amplitude of the current stimulus, which would remove the inactivation (Fig. 3B).

The impulse responses adapt in a brief period of time in the rapidly adapting neuron irrespective of amplitude and the type of stimulation (cf. Rydqvist and Purali 1993). In the rapidly adapting neuron a reduction of the current stimulus is not associated with any APs (Fig. 3C), contrary to what was observed in the slowly adapting neuron (Fig. 3B). However, hyperpolarization improves firing duration (Fig. 4) mainly because inactivation is removed 10–15% (cf. Fig. 10), which is sufficient to change the firing behavior considerably. The nonadapting behavior of the impulse responses when sinusoidal current stimulation was added also supports the idea that even small oscillations in the fraction of noninactivated sodium channels may affect firing behavior. When the cells were exposed to ScVLq the $h_\infty$ curve was shifted toward more positive potentials (Fig. 12, $B_a$ and $B_b$), and as expected the impulse response in the rapidly adapting neuron adapted more slowly (Fig. 6B).

In addition to the differences in the inactivation parameters, the $I-E$ relationship of the sodium current is different in the rapidly and the slowly adapting neurons. The average maximum peak current amplitude is significantly smaller in the rapidly adapting neuron. This indicates that there are fewer sodium channels in the rapidly adapting neuron compared with the slowly adapting neuron. Furthermore, the maximum sodium current in the rapidly adapting neuron is evoked by depolarizing pulses to about −45 mV, whereas in the slowly adapting neuron the maximum peak current amplitude is obtained at voltage steps to about −20 mV. However, the sodium current starts to activate at a similar potential level (also in concord with the similar threshold values, Fig. 8). Additionally, in the slowly adapting neuron the sodium current to some voltage steps has two clear max-
ima, which could be separated by a depolarizing conditioning pulse to −40 mV (Fig. 9A). The conditioning pulse shifted the I–E curve toward more positive potentials, and the difference of the peak current responses with and without a conditioning pulse gave an I–E curve similar to that observed in the rapidly adapting neuron (cf. Figs. 8 and 9B). The potential for maximum sodium current is shifted 5–10 mV toward more positive voltage. Alternatively, the current components could as well be revealed by voltage clamping the cells with voltage ramps (Figs. 9C and 12C). Because the geometry and passive properties of the two neurons are similar (Rydqvist and Purali 1991), space-clamp differences cannot be the cause of the two Na⁺ current maxima observed in the slowly adapting neuron. The problem would be greater in the rapidly neuron because its axon was pinched further out.

The low-threshold component has fast activation and inactivation properties. The result shown in Fig. 2B indicates that in the rapidly adapting neuron the low threshold current component, constituting most of the total sodium current, may be located in the axonal region. This component is also present in the slowly adapting neuron at a similar magnitude (cf. Fig. 9B). In contrast, the high-threshold current component has slower activation and inactivation properties with a different voltage dependence (Figs. 7 and Fig. 9). This component is either small or absent in the rapidly adapting neuron (Figs. 9 and 12), which is most apparent difference between the sodium current in the rapidly and the slowly adapting neurons. Perhaps the difference leads to other differences both in the kinetics of the sodium currents and in the resulting APs and impulse responses. The presence of the high-threshold current component in the slowly adapting neuron may as well be the cause of the rhythmic depolarization amplitudes induced by ScVlq only in the slowly adapting neuron at resting potential (Fig. 6C).

The properties of the sodium current are altered by ScVlq (Fig. 12). The amplitude of the sodium current is increased, the rate of inactivation is slowed, the steady state of inactivation (hn) curve is shifted toward more positive potentials, and the I–E plots of the peak sodium current show an increase in current amplitude in both the rapidly and the slowly adapting neurons in agreement with earlier studies on frog myelinated nerve and muscle (Catterall 1979; Koppenhöfer and Schmidt 1968; Strichartz and Wang 1986). However, the changes induced in the I–E plots are different so that the hump observed in the slowly adapting neuron becomes more prominent (Fig. 12Ab). The difference may be due to superimposed current components; when inactivation is slowed down the high-threshold component may completely overlap the low-threshold component (Fig. 12C). The findings indicate that both components are affected by ScVlq. However, the slowing of the inactivation of the high-threshold current component may be somewhat less compared with what is seen in the low-threshold component (Fig. 12C).

The inward current carried by sodium ions is present both in the rapidly and the slowly adapting receptor neurons. However, the amplitude, I–E relation, inactivation properties, and some pharmacological properties of the sodium current differ between the two types of receptor neurons. This indicates that there might be several types of sodium channels in the two types of neuron and a possible difference in the spatial distribution of the channels that can account for the differences both in the individual APs and in the impulse responses to electrical and mechanical stimulation. It is also conceivable that the impulse generation site is situated at different locations in the axons of the slowly and rapidly adapting neuron, which could also affect impulse responses. Sodium permeability systems composed of several different sodium channels are present in sensory neurons (Caffrey et al. 1992), in skeletal and smooth muscle fibers (Haimovich et al. 1987; Muraki et al. 1991), and in heart muscle cells (Ju et al. 1996). Future studies should include a more detailed analysis of the activation properties of the inward sodium current and a modeling of the impulse response to stretch and electrical stimulation (Swerup and Rydqvist 1996) to confirm the adaptive differences between the two receptors.

We are grateful to Drs. Joe Bruton and Christer Swerup for fruitful comments on the manuscript. The expert help of M. Tunberg-Eriksson is greatly appreciated.

This study was supported by grants from the Swedish Medical Research Council Project 6838, Karolinska Institutet, and The Scientific and Technical Research Council of Turkey.

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Received 5 February 1998; accepted in final form 9 July 1998.

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