CHAPTER 2

Ion Channels for Mechanotransduction in the Crayfish Stretch Receptor

Bo Rydqvist
Department of Physiology and Pharmacology, Karolinska Institutet,
SE-171 77 Stockholm, Sweden

I. OVERVIEW

Mechanosensitivity is found in almost every cell in all organisms from bacteria to vertebrates and covers a wide spectrum of function from osmosensing to mechanical sensing in the specialized receptors like the hair cells of the cochlea. The molecular substrate for such mechanosensitivity is thought to be mechanosensitive ion channels (MSCs). Since most development regarding the molecular aspects of the MSC has been made in nonsensory or sensory systems which have not been accessible to recordings from ion channels, it is important to focus on mechanosensitivity of sensory organs where their functional importance is undisputed. The stretch receptor organ

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(SRO) of the crustaceans is a suitable preparation for such studies. Each organ contains two receptors: one slowly and one rapidly adapting receptor neurons. The primary mechanosensitivity is generated by two types of MSC of hitherto unknown molecular type located in the neuronal dendrites, which are inserted into a receptor muscle fiber. In addition to the MSCs, the neurons contain voltage-gated Na^+ channels which seem to be differently located in the slowly and rapidly adapting neurons. Finally, at least three types of voltage-gated K^+ channels are present in the sensory neurons, the location of which is not known. The spatial distribution of ion channels and the kinetics of the channels, together with the viscoelastic properties of the receptor muscles, determine the overall transducer properties and impulse firing of the two receptor neurons including their typical adaptive characteristics.

II. INTRODUCTION

The crustacean SRO has been a major preparation for the study of mechanotransduction both on the macroscopic and on the ion channel levels. The SRO is considered to be an organ analogous to the mammalian muscle spindle organ that is instrumental for proper skeletal muscle function. The receptor organ was first described in the lobster by the Polish-British zoologist Alexandrowicz (1951, 1967). Later, Florey and Florey (1955) described the same type of organ in the crayfish (*Astacus fluviatilis* presently named *Astacus astacus*). Identical and similar muscle receptor organs can also be found in a number of other invertebrate phyla such as Mollusca, Chelicerata, and Uniramia (for a review see Rydqvist, 1992). The importance of this organ, and its accessibility relative to the human muscle spindle, and mechanotransduction in general, was soon acknowledged and triggered a number of electrophysiological studies in several laboratories (Wiersma *et al.*, 1953; Kuffler, 1954; Eyzaguirre and Kuffler, 1955a,b; Edwards and Ottoson, 1958).

The mechanosensory neurons of the SRO of the crayfish are of the nonciliated type and are different from the ciliated type represented by the classical hair cells in the hearing organs. Most investigations regarding the molecular aspects of the MSC have been made in nonsensory systems, and it is thus important to focus on mechanosensitivity of sensory organs where the functional importance of these channels is undisputed. The SRO of the crustaceans is a suitable preparation for such studies. The SRO is experimentally accessible to mechanical manipulation and electrophysiological recordings using intracellular microelectrodes or patch clamp techniques for ion channel analysis, although the latter technique is not without problems since the sensory neuron is covered by supporting glial cells. It is,

however, relatively easy to inject substances into the neuron, which makes the neuron accessible to measurements using fluorescent probes.

In the present chapter, I have focused on the overall function of the SRO in the crayfish stretch receptor including results obtained with structural techniques, classical electrophysiology, and patch clamp techniques. The main emphasis will be on the mechanotransduction processes and the ion channels involved in the SRO of the species *A. astacus*, *Pacifastacus leniusculus*, *Procambarus clarkii*, and *Orconectes limosus*.

III. MORPHOLOGY OF THE SRO

Since the crayfish stretch receptor is a genuine mechanosensory organ with several ion channels involved in the overall mechanotransduction, a brief description of the SRO seems relevant. The SRO has two sensory neurons, each connected to a receptor muscle, located in the extensor muscles of the abdomen (Florey and Florey, 1955; Purali, 2005). The sensory neuron is of the multipolar type (Fig. 1) with its dendrites inserted into the central (intercalated) part of the receptor muscle which consists of only one muscle cell (Tao-Cheng *et al.*, 1981). The receptor muscles insert on consecutive segments and the afferent axons from the neurons join the dorsal segmental nerve to the ventral ganglion. The SROs also receive efferent innervations: (1) one or two motor axons to the receptor muscle cell and (2) two or three accessory axons conveying inhibitory signals to the receptor muscle and the sensory neurons (Alexandrowicz, 1951, 1967; Elekes and Florey, 1987a,b). Functionally, the receptors are activated when stretched by flexion of the abdomen or contraction of the receptor muscles (Kuffler, 1954) and the SROs are involved in the control of the extensor muscles.

In the crayfish, both the slowly and the rapidly adapting receptor muscles consist of a single muscle fiber that is divided by invagination of the cell membrane into numerous cytoplasmic processes in the central region of the muscle, the intercalated tendon, which is mainly made up of collagen. Some of the myofibrils insert in the intercalated tendon but some pass this region. The slowly adapting muscle is in the order of 30–80 μm in the central region but considerably thinner in the distal ends. The rapidly adapting muscle has a more even diameter and is thicker 70–150 μm (Komuro, 1981).

The sensory neurons are large (30–100 μm) multiterminal cells of mainly pyramidal or fusiform shape. They contain a nucleus (ca. 10 μm) with a clear nucleolus. The dendrites branch about four to five times and intermingle with the connective tissue and muscle strands in the intercalated tendon. The fine terminal branches are about 2-μm long and about 0.1 μm in diameter and are devoid of mitochondria (Tao-Cheng *et al.*, 1981). The axon is in the

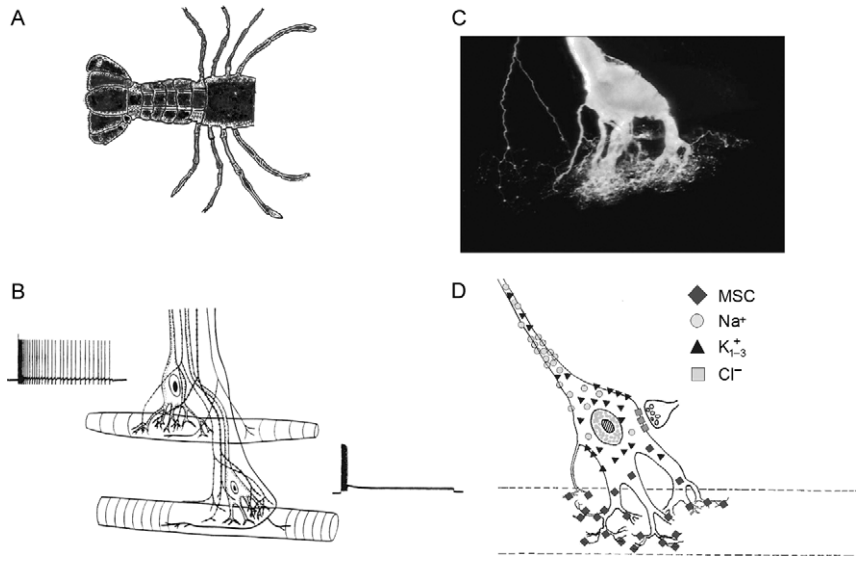

FIGURE 1 (A) Abdomen and thorax of crayfish. (B) Slowly (top) and rapidly (bottom) adapting receptor with typical action potential firing pattern as a result of a ramp and hold extension of the receptor muscle. (C) Confocal microscopic image of a slowly adapting neuron injected with Fluo-4 (Bruton and Rydqvist, unpublished data), a recurrent fiber is present at left. (D) Drawing of stretch receptor neuron with proposed channel distribution. (A, B) Adapted with kind permission from Springer Science and Business Media (Rydqvist, 1992).

order of 30 μm in diameter. The receptor neurons of the crayfish have several layers of sheet cells that surround them except for the dendritic tips.

The fine structure of the inhibitory synapses has been investigated by several authors (Elekes and Florey, 1987a,b) using serial sectioning and immunohistochemical technique, which have revealed a complex array of GABAergic inhibitory synapses on the axon, neuron, and muscle fibers and also reciprocal synapses on the inhibitory axon.

IV. FUNCTIONAL PROPERTIES

A. General Behavior

The receptors are activated (stretched) by flexion of the abdomen or contraction of the receptor muscle. The receptors are involved in the motor control of the abdominal muscles and the physiological range is up to 40% of resting length (Alexandrowicz, 1951). The first measurements from the

receptor neuron were done by Wiersma *et al.* (1953) and Kuffler (1954) and subsequent studies by Eyzaguirre and Kuffler (1955a,b) and Edwards and Ottoson (1958) on lobster and crayfish showed that stretching the receptor organs gave rise to a distinctive pattern of impulse discharge from the neurons. It was found that the firing properties of the two neurons were clearly different, one neuron maintained firing as long as the stretch was applied (slowly adapting) whereas the other neuron generated a short high frequency discharge (rapidly adapting) at the onset of the stretch (Fig. 1B).

The chain of events that leads from extension of the receptor muscle to action potential generation in the stretch receptor is represented by the steps outlined in Fig. 2. In a first step, the extension of the receptor muscle is

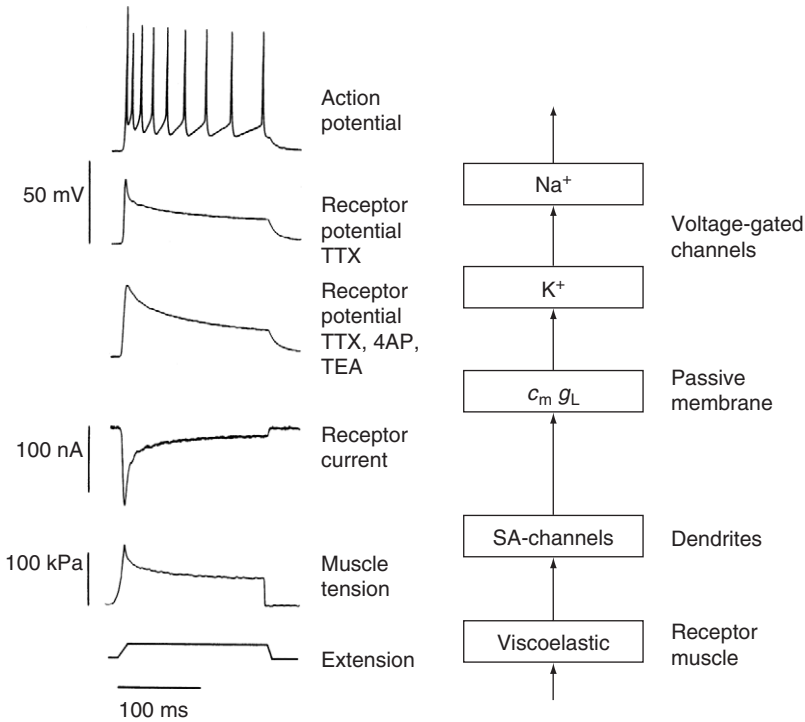

FIGURE 2 Transduction processes in a stretch receptor neuron. Left: recorded responses of muscle tension, receptor current, receptor potential, and action potentials in response to a ramp and hold extension of the muscle. The receptor potential is seen both after block of Na^+ channels with tetrodotoxin (TTX) and after additional block of K^+ channels with tetraethylammonium chloride (TEA) and 4-aminopyridine (4-AP). Right: functional blocks in transduction. Stretch-activated channels; SA channels, MSC. Adapted from Swerup and Rydqvist, 1992 with permission from Elsevier.

converted to tension in the muscle, which leads to deformation of the dendritic membrane of the sensory neuron. This opens nonselective mechanosensitive (gated) ion channels (MSCs) permeable to Na^+, K^+, and Ca^{2+} ions producing an inward generator current (Erxleben, 1989). The transformation from generator current to impulse response is a complex process determined by the passive membrane properties, that is capacitance (c_m) and membrane resistance (r_m or leak conductance g_L), and the voltage-gated ion conductances (g_{ion}) present in the neuron. At present only voltage-gated K^+ and Na^+ channels and a Ca^{2+}-activated K^+ channel have been observed. Figure 2 shows the receptor potential after block by tetrodotoxin (TTX), 4-aminopyridine (4-AP), and tetraethylammonium chloride (TEA), and the receptor potential after block with TTX only. In addition, the geometry of the cell and the spatial distribution of the different ion channels will contribute to the type of impulse response seen in the cell. The difference in responses reflects the relativity of the concept of receptor potential (see discussion in Swerup and Rydqvist, 1992).

B. Viscoelastic Properties of the Receptor Muscles

In most mechanosensory cells, the accessory structures contribute to the overall behavior of the transducer function. In particular, it has been discussed to what extent the accessory structures contribute to the adaptation in sensory cells. This is obvious in, for example, the Pacinian corpuscle. The two neurons in the SRO have different adaptive properties and this could arise solely from possible differences in passive mechanical properties in the two receptor muscles. Earlier studies (Kuffler, 1954) observed that the contractile properties indeed differed; the rapidly adapting muscle had properties resembling a fast twitch fiber, whereas the slowly adapting muscle behaved as a slow twitch fiber. The viscoelastic properties of the receptor muscles in the slowly and rapidly adapting receptors were investigated by extending the muscles while measuring the resulting force at one end of the muscle fiber (Rydqvist *et al.*, 1991, 1994). It was found that the viscoelastic properties of the two muscles differed considerably, the rapidly adapting receptor muscle having more dynamic characteristics (Fig. 3A). The muscles could be reasonably well described by a viscoelastic model consisting of a Voigt element (parallel spring and damping element, Fig. 3, inset) in series with a nonlinear spring (Rydqvist *et al.*, 1991; Swerup and Rydqvist, 1996). The difference in viscoelastic properties probably relates to the morphological differences mentioned above. At least, part of the difference in adaptive properties between the slowly and rapidly adapting receptors is due to the different viscoelastic properties of the muscle fibers (Rydqvist *et al.*, 1994).

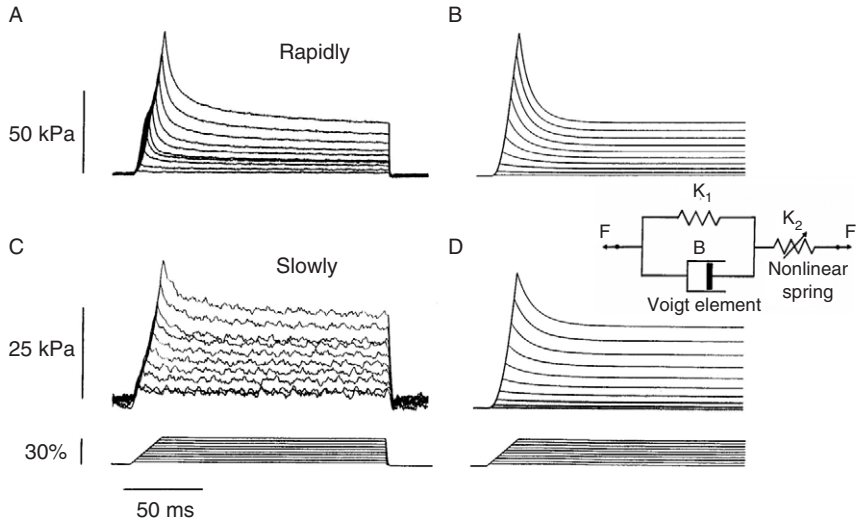

FIGURE 3 Typical tension responses due to imposed stretches in the (A) rapidly and (C) slowly adapting receptor muscle. (B and D) Calculated responses in the rapidly and slowly adapting receptor muscle, respectively, using the model seen in the inset and using different spring and dashpot values. Adapted with permission from Blackwell (Rydqvist *et al.*, 1994).

C. MSCs in the Receptor Neurons

As is evident from other chapters in this volume, considerable advances have been made in the field of structure and function of MSCs. It is now over 20 years since the first MSC, or stretch-activated (SA) ion channel, was reported and analyzed by Sachs and coworkers (Guharay and Sachs, 1984) in cultured chick embryonic muscle cells. This was soon followed by recordings from MSCs in bacteria which also gave rise to the first molecular structure of an MSC (Martinac *et al.*, 1987; Sukharev *et al.*, 1994; see also Kung, 2005). However, relatively few sensory systems have been studied in terms of MSCs despite the fact that few doubt that mechanotransduction in different sensory cells is due to opening of MSCs, for example hair cells in the hearing organ, the crayfish stretch receptor, and touch receptors in the nematode *Cenorhabditis elegans*. Recordings from single MSCs using the patch clamp technique have been made only in a few pure mechanosensory neurons due to the fact that the MSC are situated in very fine cilia and thus not easily accessible to single-channel recording. Instead, whole-cell recordings and indirect methods like knockout techniques as in *C. elegans*

and molecular techniques such as *in situ* hybridization have been used to define the presence of MSC (Sukharev and Corey, 2004).

The crayfish stretch receptor is one of the few undisputed mechanosensory organs where actual recordings and analysis of MSCs have been performed (Erxleben, 1989). On the other hand, the molecular structure of the channel is still not determined. The receptor neurons (slowly and rapidly adapting) are of the nonciliary type, which probably have implications for the type of MSC present in these neurons. The sensitivity of the crayfish MSCs is very high compared to other MSCs, for example the bacterial channels (Hamill and McBride, 1994). Erxleben (1989) reported the presence of two types of MSCs in the slowly adapting neuron of the crayfish *O. limosus*. These channels are believed to be present in high density in the extensive dendritic tree of the neuron (Fig. 1D) but are also present in the large dendrites and the soma. Since the dendrites are too small and buried in the intercalated zone of the receptor muscle, the only possible parts of the neuron accessible to patch clamp are the soma and the main dendrites. Erxleben (1989) found a marked increase in single-channel activity when the membrane of the patch was deformed by applying suction to the pipette (Fig. 4). Two different types of MSCs were reported on the soma and the primary dendrites of the neuron with similar conductance properties but different voltage range of activation and different sensitivity to membrane tension: (1) an inward-rectifying SA (RSA) channel which responded only weakly to membrane tension and (2) an SA channel which was only weakly voltage dependent but was more sensitive to membrane tension.

The RSA was inactive when no suction was applied to the pipette and showed a decreased open probability when the patch was depolarized (Fig. 3 in Erxleben, 1989). The RSA was also found mostly in the soma, whereas the SA channel was found predominantly in the large dendrites.

Figure 4A and B show single-channel recordings from RSA and SA ion channels. Whereas the RSA never reached saturation in the suction range used, the SA channel displayed a classical sigmoid relation between suction pressure and open probability (P_0) with a saturating pressure of about 25 mmHg (Fig. 4C and D). These experimental curves could be described by either of the following Boltzmann equations:

$$P_0 = \frac{1}{1 + k\ \exp(-sp)} \tag{1}$$

$$P_0 = \frac{1}{1 + k\ \exp(-sp^2)} \tag{2}$$

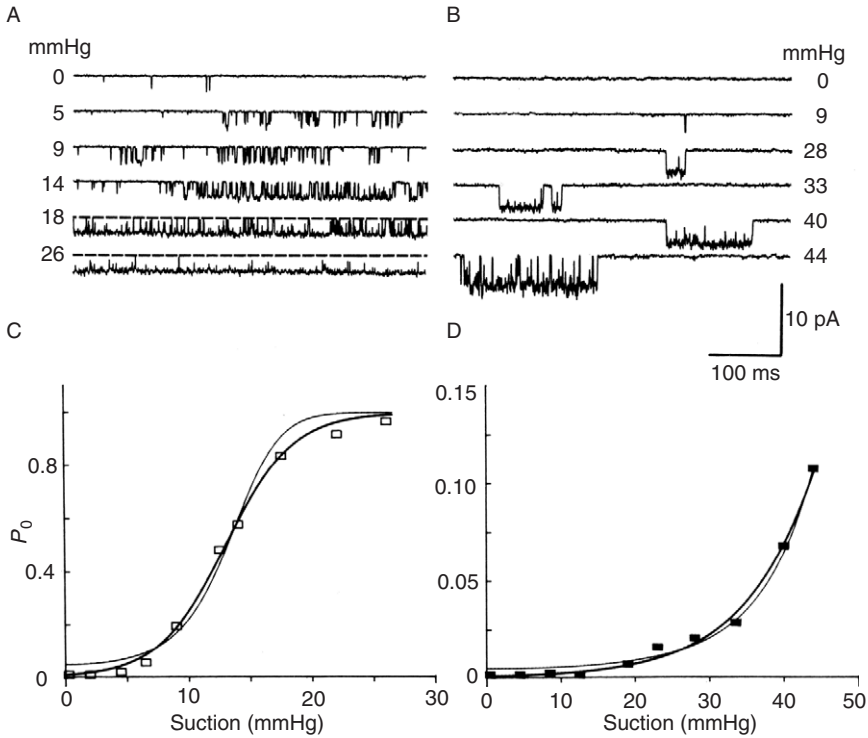

FIGURE 4 The two types of mechanosensory channels observed in the soma of the crayfish (*Orconectes limosus*) slowly adapting neuron. (A) SA (stretch-activated) type of mechnanosensitive ion channel. (B) RSA (rectifying SA) type mechanosensitive channel. (C) Stimulus–response relation of SA channel. (D) Stimulus–response relation of RSA channel. Reproduced from Erxleben (1989) by copyright permission of The Rockefeller University Press.

where k is a pressure insensitive term, s the sensitivity, p the applied negative pressure in the pipette, and P_0 the open probability of the channel (Erxleben, 1989).

The two equations illustrate the two types of gating mechanisms that have been proposed (Sokabe and Sachs, 1992; Kung, 2005). The open probability dependence of the squared suction pressure is derived from the model where the force in the lipid bilayer is changing/gating the MSC. In the linear model, the open probability is dependent on p, and is equivalent to the tethered type of gating where a spring is attached to the MSC controlling the gate.

From available experimental data, it is obvious that it is not possible to distinguish between these two models for the RSA and SA in the crayfish

sensory neurons. However, the sensitivity of RSA is close to what was found for one of the MSCs in *Escherichia coli*, the MscL, a channel that is reported to have a gating mechanism that is best described by the lipid phase model (Martinac *et al*., 1987). This stems from the fact that mechanosensitivity was preserved even when the MscL was inserted in pure lipid artificial membranes where the force acting on the channels must come from the lipid phase. The squared model was also concluded by Guharay and Sachs (1984) to best fit the gating properties of the chicken muscle MSCs. Other animal cell MSCs are, however, thought to be gated through a tether (Kung, 2005). The issue of gating regarding the SA and RSA in the crayfish neuron is at the moment an open question, and it is not possible to deduce information about the structural nature of the RSA and SA in the crayfish neurons.

The permeability properties of the crayfish stretch receptor MSC were studied by Brown *et al*. (1978) and Edwards *et al*. (1981) from macroscopic currents and by Erxleben (1989) from patch clamp recordings. Both types of studies show permeability for Na^+, K^+, and Ca^{2+} ions. The single-channel analysis gave a slope conductance for K^+ of 71 ± 11 pS, Na^+ of 50 ± 7.4 pS, and Ca^{2+} of 22 ± 3 pS. Assuming an average resting membrane potential of −70 mV, the reversal potential for the SA current was estimated by extrapolation to be about 0 mV. The permeability through the rectifying channel RSA was similar: 44 pS for Na^+ and 22 pS for Ca^{2+}. This can be compared to the estimate based on macroscopic currents. Brown *et al*. (1978) found a value for the reversal potential of 13 ± 6.5. The result was in general agreement with those reported by other groups (Obara, 1968; Klie and Wellhöner, 1973), even though these estimates were made by extrapolation. However, using Tris (Trizma) and arginine as substitute for Na^+, it was shown that even these ions could permeate the MSC in the receptor neuron and the P_{Tris}/P_{Na^+} was 0.31 and the $P_{Arginine}/P_{Na^+}$ was 0.25 (Brown *et al*., 1978). This indicates that the pore of the crayfish MSC might be of considerable size. In Edwards *et al*. (1981), the size of the channel was discussed and it was concluded that the crayfish MSC must be of about the same size or slightly larger than the acetylcholine receptor channel, that is around 7 Å.

The permeability of divalent cations was also investigated independently and based on the SA currents. The permeability for Ca^{2+} and Mg^{2+} compared to Na^+ was estimated to be 0.3 and 0.4, respectively (Edwards *et al*., 1981), similar to what was found by Erxleben (1989) (see above). In the rapidly adapting receptor $P_{Ca}/P_{Na} = 0.44$ and $P_{Mg}/P_{Na} = 0.60$ (Rydqvist and Purali, 1993). These values were based on $P_{Na}/P_K = 1.6$ and 1.5 for the slowly and rapidly adapting receptor neurons, respectively, which is slightly at odd with the values for Na^+ and K^+ conductances obtained by Erxleben (1989). The influx of Ca^{2+} was suggested by Erxleben (1993) to be responsible for part of the adaptation of the receptor current by activating a Ca^{2+}-dependent K^+ channel in the neuron. In recordings from patches containing

both SA and K_{Ca}, he was able to demonstrate that there was a concomitant increase in the open probability for the SA channel and the K_{Ca} channel. These results are in line with measurements of macroscopic stretch-induced currents (Ottoson and Swerup, 1985a,b). However, it was also found that Ca^{2+} had direct effects on the MSCs. When Ca^{2+} was reduced in the external solution from the normal concentration of 13.5 mM to 1.35 and 0.13 mM, the stretch-induced generator current increased. This was interpreted as an effect of Ca^{2+}, possibly on an internal site of the MSC, by which Ca^{2+} reduced the permeability to monovalent cations or decreased the open probability of the crayfish MSC (Brown *et al*., 1978). The effect was observed even when Na^{+} was substituted with Tris or arginine.

D. Macroscopic Receptor Currents in the Stretch Receptor Neurons

The findings at the single-channel level can be compared to the macroscopic current response to stretch, that is the receptor current. If the sensory neuron of the stretch receptor is subjected to voltage clamp, it is possible to observe the receptor current generated by extending the receptor muscle without interference of voltage-gated ion channels. This means that the receptor current reflects the activation of the MSCs of the receptor neuron. It should be observed, however, that a Ca^{2+}-activated K^{+} current (Erxleben, 1993) could be present, since the MSCs in both slowly and rapidly adapting receptor neurons are permeable to Ca^{2+} (Edwards *et al*., 1981; Rydqvist and Purali, 1993) as discussed in the previous section. However, the quantitative contribution at the macroscopic level is still unclear because using several blockers of Ca^{2+}-dependent K^{+} current did not indicate any effect of either slowly or rapidly adapting neuron (Purali and Rydqvist, 1992). In addition, it cannot be altogether ruled out that that some non-MSC could be involved in the generation of the receptor current. However, there is so far no evidence for such channels.

As is seen in Fig. 2, there is an essential difference in shape between the time course of tension and current response to a ramp and hold extension (cf. Swerup and Rydqvist, 1992), which is also seen comparing Figs. 3 and 5. This suggests that there is no simple linear relation between tension and current but that the current must be related to the tension in the receptor muscle through the SA ion channels (MSCs).

Consequently, the stimulus-response relation for both the slowly and rapidly adapting receptors is not linear but typically sigmoid in character (Rydqvist and Swerup, 1991; Rydqvist and Purali, 1993), and the amplitude of the receptor current reaches a maximum probably determined by the number of MSCs being simultaneously open (Fig. 5C). Using a log–log relationship between the stimulus and the response, the sigmoid character

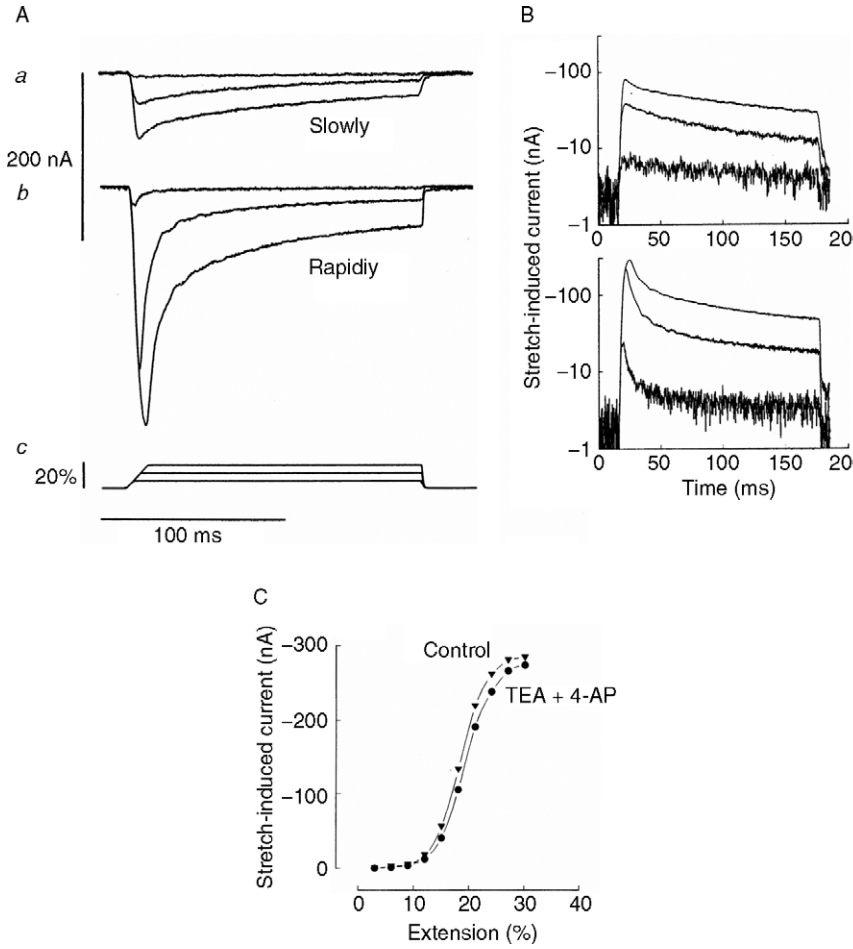

FIGURE 5 (A) Receptor currents in slowly and rapidly adapting neurons from the same organ due to ramp and hold extensions (6%, 12%, and 18%) of the receptor muscle. (B) The decay phase is quite different which is more clearly seen in the log plot in B. (C) Typical stimulus response characteristics for a rapidly adapting neuron. TEA + 4-AP did not affect the receptor current. With permission from Blackwell (Rydqvist and Purali, 1993).

of the relation was preserved indicating that a Stevens' power law is not applicable over the entire stimulus range (Stevens, 1957). For the virtually linear part of the log–log curve for the peak current (10–20% extension), the *n*-value as defined by Stevens was 3.0 in the slowly adapting neuron and 4.7 in the rapidly adapting neuron (Rydqvist and Swerup, 1991; Rydqvist and

Purali, 1993). These are very steep relationships compared to many other mechanosensory systems, indicating that in this physiological range (10–20%) the muscle receptors have a considerable impact on the reflex arcs controlling the extensor muscles of the abdomen. This type of movement is characteristic for escape responses of the crayfish. However, for small extensions, less than 10%, the slope is close to 1 which indicates that for small length changes of the extensor muscle the SRO could have a more moderate impact on the motor system. The time course of the receptor current of the slowly and rapidly adapting stretch receptors differ, the rapidly adapting receptor current having a typical dynamic character (Fig. 5A and B). This can be compared to tension development in the receptor muscles, which also displays a more dynamic character in the rapidly adapting muscle fiber as compared to the slowly adapting muscle (Fig. 3A and C).

To investigate if it was possible to calculate the receptor current and potential using available data for the SRO, Swerup and Rydqvist (1996) developed a model that took into account data from the slowly adapting stretch receptor. The model was based on viscoelastic properties of the muscle fiber, the biophysical properties of the MSCs, and the passive properties of the neuronal membrane (a lumped leak conductance and capacitance). The model could take into account a wide range of experimental data from the slowly adapting neuron provided that a time-dependent shift of open probability of the MSC (MSC adaptation) was taken into account (Hamill and McBride, 1994; see also Section IV.G).

E. Pharmacology of the Crayfish MSCs

Although a molecular characterization of the MSC in the crustacean stretch receptor has not been successful so far, despite several attempts, pharmacological characterization has given some clues to the molecular nature of the MSC (for a review on MSC pharmacology see Hamill and McBride, 1996). The MSCs of the stretch receptor neuron or the receptor current are not affected by TTX, 4-AP, or TEA (Ottoson and Swerup, 1985b, Erxleben, 1989; Rydqvist and Purali, 1993). The trivalent lanthanide gadolinium (Gd^{3+}) was found to block the stretch-induced current in the stretch receptor neuron, although it also blocked both the voltage-gated Na^+ channel and the K^+ channel to some degree which indicates that it is not completely selective for the crayfish MSC. The crayfish receptor current was more sensitive to Gd^{3+} when Ca^{2+} was lowered, indicating some competitive interaction between these two ions (Swerup *et al.*, 1991).

Several local anesthetics were found to affect the receptor current in the stretch receptor neuron (Fig. 6). Lidocaine at low concentrations facilitated

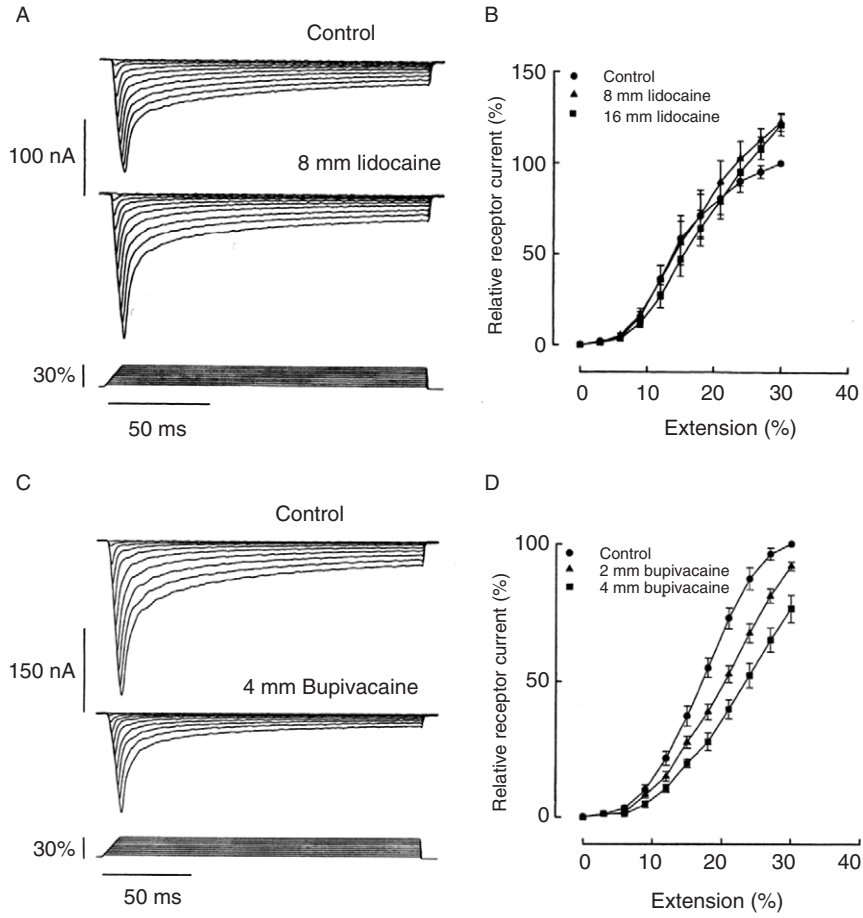

FIGURE 6 Effect of lidocaine and bupivacain on receptor currents. Stretch receptor neurons were stimulated by extensions of receptor muscle from 3% to 30% of resting muscle length. (A) Receptor currents in control solution (top) and in 8-mM lidocaine (middle) to extensions (bottom). (B) Relative peak receptor current vs extension in control solution and in 8- and 16-mM lidocaine. (C) Receptor currents in control solution (top) and in 4-mM bupivacaine (middle) to extensions (bottom). (D) Relative peak receptor current vs extension in control solution and in 2- and 4-mM bupivacaine. With permission from Blackwell (Lin and Rydqvist, 1999).

the receptor current, whereas tetracaine, bupivacaine, and an analogue to lidocaine (LL33) partially blocked the receptor current (Lin and Rydqvist, 1999a). There was a correlation between the effect on receptor current and the oil:water distribution coefficient which indicates that the local anesthetic

blocking effect is mediated through the lipid phase. In an earlier study, it was also shown that several nonionic detergents in the Triton series (i.e., Triton X-100 and X-45) could block the receptor current (Ottoson and Rydqvist, 1978). These results suggest that this particular MSC can be modulated through the lipid phase and that the gating mechanism is similar to what is found for bacterial MSCs (Martinac, 2004).

Some transient receptor potential (TRP) ion channels have been suggested to be mechanosensitive (Martinac, 2004; Sukharev and Corey, 2004; Kung, 2005), for example the TRPV4, TRPA1, TRPN. This is an attractive possibility since the TRPN channel family (NompC) was shown to be responsible for mechanosensitivity in *Drosophila*—an invertebrate relative (Walker *et al*., 2004). We have used ruthenium red, known to block TRP channels, to investigate possible effects on the stretch receptor neuron. However, no effect on the receptor current in the stretch receptor neuron has been observed (Fernström and Rydqvist, unpublished observations). Ruthenium red is probably a crude tool in this respect, and considering the great number of possible TRP channels involved in mechanosensing these results must be interpreted with caution.

A spider toxin (GsMTx4) from the spider *Grammostola spatulata* has been shown to block some MSCs in the heart (Bode *et al*., 2001), astrocytes, and kidney (Suchyna *et al*., 2000). In preliminary experiments, we have studied the effects on the receptor current of the purified fraction GsMTx4 at concentrations up to 10 μM. Only minor effects on the receptor current were found using the toxin (Fernström, Rydqvist, and Sachs, unpublished observations).

In a similar type of experiment, the stretch receptor preparation was exposed to 1 mM amiloride, a substance known to block MSCs of the ENaC/DEG type (epithelial Na^+ channel, degenerin channel protein), that are responsible for mechanotransduction in *C. elegans* and hair cells (Charfie and Sulton, 1981; Driscoll and Chalfie, 1991; Martinac, 2004; Sukharev and Corey, 2004). Our results clearly show that this substance has very small effects on SA currents, since in three experiments no significant effect could be demonstrated (Fernström and Rydqvist, unpublished observations).

It is thus an open question as to what are the molecular constituents of the MSC in the crayfish stretch receptor neuron. It is observed in experiments using local anesthetics and detergents on the stretch receptor that substances that perturb the lipid phase have an increased tendency to affect mechanotransduction. Further, the more hydrophobic the substance is the larger the blocking effect. This indicates that the MSC in the sensory neuron is gated through the lipid phase and not through the cytoskeleton or extracellular matrix, that is a tethered model. This is supported by the fact that the local anesthetic shifted the stimulus–response curve indicating an effect on gating

(Lin and Rydqvist, 1999a) and not simply a plugging of the ion channel pore. In addition, the relatively slow onset of the effect in these experiments, similar to what was found by Martinac *et al.* (1990), could be explained by diffusion of the anesthetics into the lipid bilayer.

F. Voltage-Gated Ion Channels and the Generation of Impulse Response

1. Na^+ Channels

Like many other neurons, the action potential or nervous impulse in the stretch receptor neuron is generated by Na^+ and K^+ ion channels. No voltage-gated Ca^{2+} channel has been implicated in this process. Generation of action potentials is fundamental in shaping the final mechanotransduction response in the sensory neuron and the properties of the ion channels involved in this process have important consequences for the particular type of response seen in the rapidly and slowly adapting neurons. It is thus not surprising that the shape of action potentials in the slowly and rapidly adapting neurons differ. In the rapidly adapting receptor neuron the amplitude is around 55 mV, whereas in the slowly adapting neuron the amplitude is around 80 mV. It was also found that the duration of the action potential was longer in the slowly adapting receptor mainly due to a slower repolarization (Purali and Rydqvist, 1998). This is consistent with the difference in properties of the Na^+ and K^+ channels. Na^+ currents generate action potentials in both the slowly and rapidly adapting neurons. In the slowly adapting neuron, the Na^+ current is larger and the inactivation (τ_h) is slower and takes place at more negative potentials compared to that in the rapidly adapting neuron, consistent with the properties of the action potentials. It was also observed that pinching the axon of the rapidly adapting neuron close to the soma totally abolished the action potentials, contrary to what was found in the slowly adapting neuron. Further, in the rapidly adapting neuron, the action potentials recorded in the axon were larger and had slightly faster rise time than those recorded in the soma (Purali and Rydqvist, 1998). Taken together, these observations point toward a possible difference in Na^+ channel distribution in the two types of neurons (Fig. 7B).

Recordings of Na^+ currents in the slowly adapting neuron using the two-electrode voltage clamp technique indicated the presence of two different Na^+ channel populations with different kinetic properties (Purali and Rydqvist, 1998; Lin and Rydqvist, 1999b). Since this was not the case using macropatch clamp recordings in the soma of the slowly adapting neuron (Lin *et al.*, 1999), the observations point toward a specific spatial distribution of at least two different sets of Na^+ channels. As a result of these

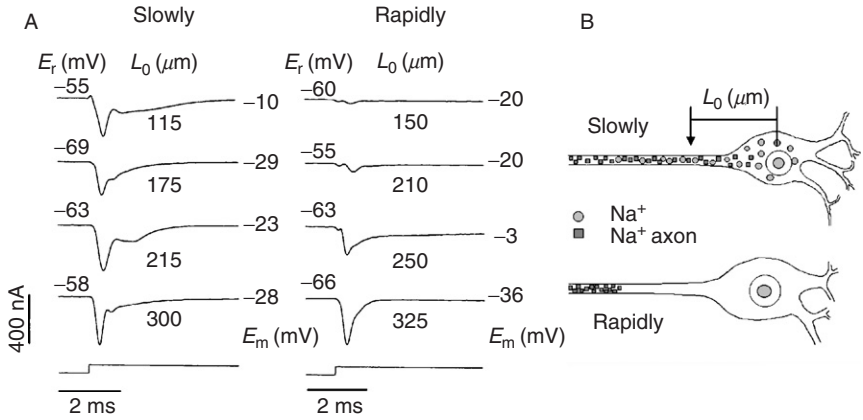

FIGURE 7 (A) Peak Na^+ currents from four slowly and four rapidly adapting neurons in which the axon was cut as indicated in B. L_0 indicates distance from center of nucleus to cut position. E_r is resting membrane potential, which was also holding potential. E_m is the potential value for peak Na^+ current. (B) Suggested Na^+ channel distribution in the slowly and rapidly adapting neurons. Two types of Na^+ channels are proposed, one (Na^+) that can be present in both soma and axon and one (Na^+-axon) present in axon only. Arrow indicates location of cutting the axon as stated in B. Reprinted from Lin and Rydqvist (1999) (A) with permission from Elsevier, and from the author (Lin, 2000) (B).

observations, we recorded Na^+ currents from the soma of slowly and rapidly adapting neurons after cutting the axons at different positions from about 350 to 100 μm from the soma. To our surprise, in the rapidly adapting neuron, the Na^+ current was completely abolished when the axon was cut at about 150 μm from the soma, whereas in the slowly adapting neuron most of the Na^+ current was preserved even if the axon was cut as close as 100 μm from the soma (Fig. 7).

This indicates that in the slowly adapting neuron, one of the suggested Na^+ channels is dominating in the soma but both channels may be present in the axon. In the rapidly adapting neuron, the results so far indicate a single Na^+ channel population located at least 150–200 μm out in the axon (Lin and Rydqvist, 1999b). However, there appears to be a part of the axon even further out with a high density of Na^+ channels, constituting a trigger area for the action potential as indicated from recordings of action potentials made concomitantly in the axon and the soma (Purali and Rydqvist, 1998). The difference in spatial distribution of the Na^+ channels between the two neurons suggests that it might be important for the difference in adaptation. This will be discussed further in a later section.

2. K^+ Channels

There are presently over 100 genes coding for K^+ channel subunits, including the β-units modulating K^+ channels. Many neurons and other cells contain a large number of different K^+ channels. In the stretch receptor neuron of the crayfish, three types of voltage-gated K^+ channels and one Ca^{2+}-activated K^+ channel have been identified to date.

In experiments using two intracellular electrode voltage clamp, it was shown that both the slowly and rapidly adapting receptor neurons contained at least two different K^+ channels (Rydqvist and Zhou, 1989; Rydqvist and Purali, 1991; Purali and Rydqvist, 1992). The whole-cell K^+ currents in the slowly (Brown *et al*., 1978; Rydqvist and Zhou, 1989) and the rapidly adapting neurons (Rydqvist and Purali, 1991) were characterized by a transient and an outwardly rectifying component. The activation time constant for the K^+ current in the rapidly adapting neuron was smaller and the activation took place at more negative potentials as compared to the slowly adapting receptor. The results were supported by macropatch recordings from the soma of the slowly adapting neuron which gave almost identical results (Lin *et al*., 1999). The inactivation as derived from whole-cell currents had two time constants in both receptors, a fast component of about 0.5 ms and a slow component ranging from 2 to 8 s (Brown *et al*., 1978). Pharmacological dissection of the K^+ currents in the slowly and rapidly adapting neurons using 4-AP and TEA suggested two different populations of ionic channels: one channel having high affinity to TEA and the other low affinity to TEA. The results further indicated that the low TEA affinity channel dominated in the slowly adapting neuron, whereas in the rapidly adapting neuron both channels were equally common (Purali and Rydqvist, 1992).

Later experiments using patch clamp recordings from the slowly adapting neuronal soma have demonstrated the existence of three different types of K^+ channels in this neuron (Fig. 8).

First, an outward delayed rectifier has been analyzed in detail having a single-channel conductance of 13 pS and a $P_K = 6.5 \times 10^{-14}$ cm^3/s (mean values) with little inactivation (Figs. 8A and 9). First latency analysis suggested a two closed states preceding two open states. The channel displays properties similar to a K^+ channel of the Kv1.2 type (Lin and Rydqvist, 2001). Second, a K^+ channel with large conductance (53 pS) having properties suggesting a delayed outward rectifier with some inactivation as seen from cell-attached recordings (Fig. 8B). The third K^+ channel is clearly a transient K^+ channel (Fig. 8C) with fast inactivation (estimated time constant in the order of 20–50 ms). This channel has a conductance of 23 pS. The 23 and 53 pS K^+ channels are difficult to detect at resting membrane potential but could be activated at a depolarization of 10–20 mV. Other K^+ channels with single-channel conductance of less than 10 pS have also been observed but further experiments are necessary to analyze these currents.

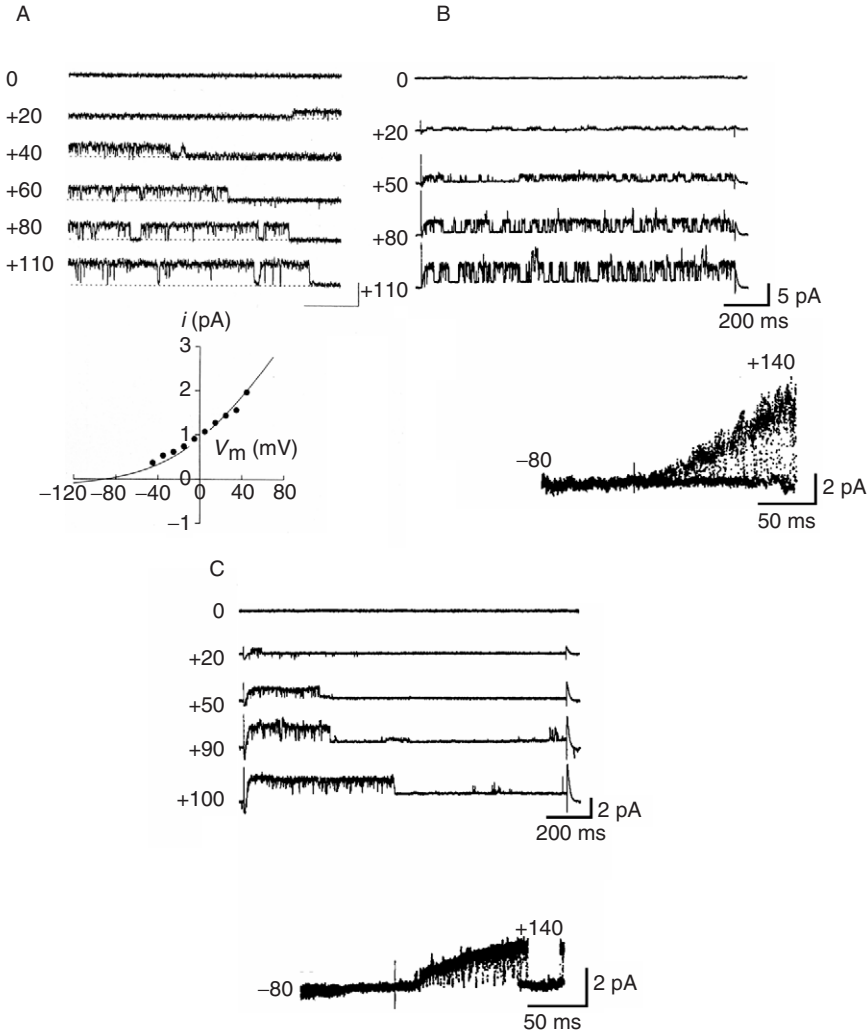

FIGURE 8 Top row: cell-attached patch clamp recordings from three different K^+ channels in slowly adapting stretch receptor neurons. The patches were depolarized from resting level to the potential indicated; normal saline in the pipette. Bottom row: *I–V* curves for the K^+ channels. In A, the curve was based on single-channel activity at potential steps; in B and C, voltage ramps were used. (A) Recordings from a 13-pS K^+ channel. In this cell, the PK $= 5.4 \times 10^{-14}$ cm^3/s (*I–V* curve bottom A). (B) Top: single-channel currents recorded in cell-attached patch from a 53-pS K^+ channel; representative currents at the potentials (marked on the left) to which the patch was depolarized from the resting state. The voltage step started and ended as indicated by the capacitative current. The bottom panel shows superimposed currents activated by voltage ramp from −80 to +140 mV; conductance 53 pS. (C) Same as in B; this K^+ channel is typical transient with conductance of 23 pS. Reprinted from Lin and Rydqvist (1999) with permission from Elsevier and the author Lin (2000, Fig. 7, p. 25).

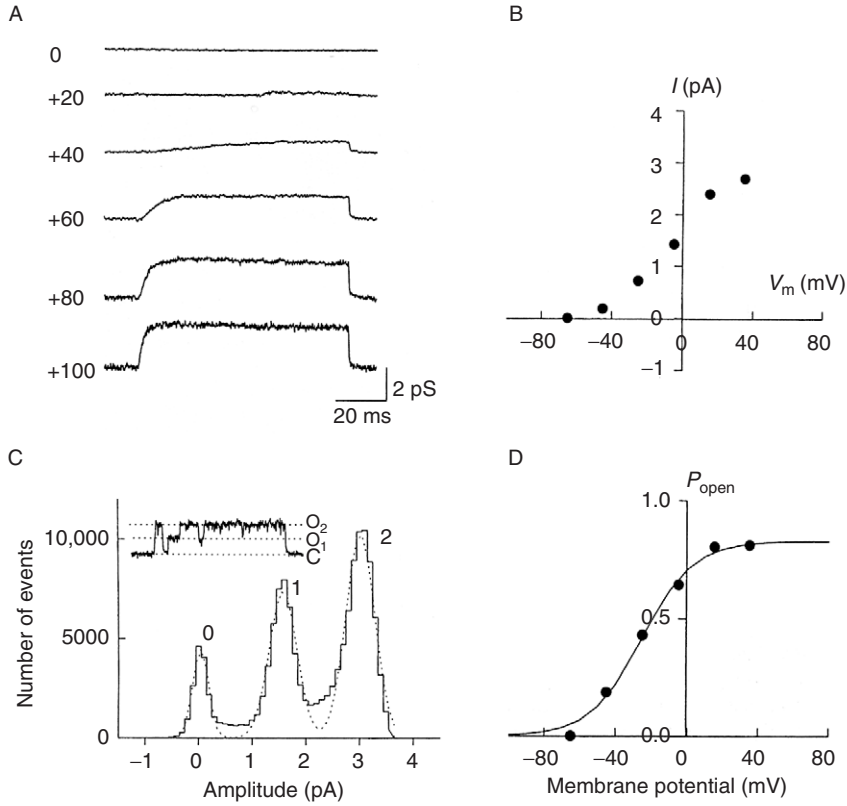

FIGURE 9 (A) Analysis of ensemble average currents from the same patch containing two K^+ channels. (A) Average currents initiated by depolarizations from 0 to +100 mV from resting membrane potential. The number of sweeps was from 105 to 194. (B) Voltage dependence of the average currents plotted against membrane potential. (C) Amplitude histogram from the recording at 80-mV depolarization (inset) and fitted to a third-order Gaussian distribution. The peaks correspond to closed level (0), one channel open (1), and two channels open (2). (D) Open probability vs membrane potential using data from A and single-channel data (C) Open probability, $P_0 = I/(i \times N)$, where I is the average current, i is single-channel current, and N the observed maximal number of channel in the patch (two in this patch). The smooth line is a fit to a Boltzmann equation. Reprinted from Lin and Rydqvist (2001) with permission from Elsevier.

It is thus clear that at least the slowly adapting neuron contains up to six different K^+ channels and that there might be considerable differences compared to the rapidly adapting neuron. This would explain part of the difference in action potential properties between the two neurons and the firing properties of the two neurons.

The spatial distribution and density of the K^+ channels have been difficult to define but experiments using macropatch recordings from different locations in the soma have given some clues (Lin *et al.*, 1999). The experiments show that the K^+ current density is highest close to the axon hillock area as compared to current densities in the dendritic part of the soma. This suggests that K^+ channel distribution is different over the neuronal surface and it can be anticipated that the slowly and rapidly adapting neurons differ in their K^+ channel distribution.

Finally, a Ca^{2+}-activated K^+ channel was suggested by Ottoson and Swerup (1982, 1985a,b) who injected EGTA and TEA into the slowly adapting sensory neurons and found changes in receptor potential adaptation consistent with effects on a K^+ channel. This was later confirmed by Erxleben (1993) who recorded simultaneously from SA (MSCs) and K^+ channels. He observed that when SA channels were stimulated by suction in the patch pipette, the K^+ channel in the same patch increased its activity. Since the K^+ channels in isolated patches were not activated by suction, he concluded that Ca^{2+} entering through the SA channel activated a nearby K^+ channel and thus was a Ca^{2+}-activated channel. This is a strong support for the suggestion made earlier by Ottoson and Swerup (1985a,b) that this channel contributed to the early adaptation in the receptor potential.

G. Adaptation: A Multifactor Property

The difference in adaptive properties between the two neurons in this receptor, as well as in other similar organs, has been a constant challenge. Figure 1B illustrates the distinct difference in adaptation of impulse discharge in responses to mechanical stimulation between the rapidly and slowly adapting receptors (cf. Rydqvist and Purali, 1993; Fig. 1). The rapidly adapting receptor gives a brief impulse discharge in response to a ramp and hold extension, whereas the slowly adapting receptor gives a sustained impulse discharge for the same stimulus. As already observed by Nakajima and Onodera (1969a,b), the same difference in impulse response is seen when the neurons are electrically stimulated (Rydqvist and Purali, 1993). Adaptation must therefore be a consequence of several processes in the receptor organs.

As can be observed from Fig. 2, the viscoelastic properties of the receptor muscle must contribute to adaptation since the tension response to ramp and hold extensions of the muscle adapts (Rydqvist *et al.*, 1991, 1994). Further, a distinct difference is seen between the tension response in the rapidly and slowly adapting receptor muscles (Fig. 3). The transient peak is more pronounced in the rapid muscle compared to that in the slow muscle.

This correlates well with the difference in receptor current seen in Fig. 5A. The receptor current of the rapidly adapting neuron has a dynamic phase that is more pronounced as compared to the slowly adapting neuron. The fast initial decay phase of the rapidly receptor current is also considerably faster as compared to that of the slowly adapting current (Fig. 5B). From studies of the viscoelastic properties and the properties of the MSCs, Swerup and Rydqvist (1996) developed a model of the primary transduction process which gave a reasonable fit to receptor potential responses of the slowly adapting stretch receptor. To achieve the fit, it was, however, necessary to include an MSC-specific adaptation originally proposed by Hamill and McBride (1994) who observed this adaptation in oocyte MSCs. This type of MSC adaptation has not been experimentally observed in the crayfish stretch receptor neuron but from the model fit it is assumed to be present in the crayfish MSC. Due to lack of quantitative data on Ca^{2+}-dependent K^+ currents (cf. Erxleben, 1993), a K_{Ca} current was not included but could be the additional factor which would make the fit even better. Differences in density of Ca^{2+}-dependent K^+ channels in the slowly and rapidly adapting neurons could be a factor determining the adaptation in receptor current.

However, since electrical stimulation gave the same principal type of adaptation in impulse discharge in the slowly and rapidly adapting neurons as seen for extension of the receptor muscle, nonmechanical factors must also contribute to adaptation. Analysis of both Na^+ and K^+ currents in the slowly and rapidly adaptive neurons as outlined above have revealed some differences in kinetic properties between the two neurons. Some of these kinetic changes are consistent with the adaptive properties seen in the two neurons. In an analysis of the Na^+ currents in the slowly and rapidly adapting neurons, it was found that the inactivation parameter in the rapidly adapting neuron was moved in the negative direction (Purali and Rydqvist, 1998) that is compatible with inactivation of impulses occurring at less depolarized levels than in the slowly adapting neuron. In a simple model of the neurons using a Hodgkin-Huxley modeling of the voltage-gated channels (Rydqvist and Swerup, 1991; Rydqvist *et al.*, 2003), it was shown that a minor change of the activation (shift of +8 mV in m parameter) and inactivation rate constants (shift of −8 mV of h parameter) could dramatically change the firing pattern in the neuron. An additional factor is the spatial distribution of the Na^+ and K^+ channels in the two neuron types. This is suggested but not proved to be an important factor for the typical pattern of impulse discharge seen in these neurons. So far, it has only been possible to define tentatively the spatial distribution of the Na^+ channels in the two neurons. As described above (Fig. 7), the rapidly adapting receptor neuron seems to have few Na^+ channels in the soma and a high concentration of the Na^+ channels in the axon, whereas the slowly neuron seems to have a similar density of Na^+ channels

in both axon and soma (Lin and Rydqvist, 1999b). It is possible that the distribution and density of K^+ channels are even more important but so far this has not been possible to determine. To ascertain the relation between spatial distribution of the different channels and the impulse response characteristics, it is necessary to develop a compartmental model of the neuron. Presently, we are in the process of developing such a model to be able to better study how different densities and distribution of channels influence impulse discharge in these receptor neurons. It will also be necessary to develop more efficient methods (e.g., *in situ* hybridization) to determine experimentally the location of ion channels on the cell membrane.

V. SUMMARY AND DISCUSSION OF FUTURE RESEARCH DIRECTIONS

The crayfish SRO is a very useful preparation for the study of mechanotransduction in all its aspects. It is a true mechanosensor, analogous to the vertebrate muscle spindle, with a clear-cut function to monitor muscle length through activation of a sensory neuron equipped with MSCs that are proposed to generate the receptor current (stretch-induced current). The receptor current activates voltage-gated Na^+ and K^+ ion channels present in the soma and axon, generating the final output of the organ: an impulse train that will reach the crayfish central nervous system. All channels thus contribute to the performance of the sensory organ. At this point, some caution should be expressed regarding the casual relation between MSC and the stretch-generated current. Even though there is no indication, to the contrary it cannot be entirely excluded that some other channels could contribute to the stretch-induced current.

The MSCs have a small conductance as compared to the bacterial MSCs (MscL and MscS). The gating mechanism is not defined, but from pharmacological results could well be due to tension in the membrane lipids, that is, the quadratic model similar to what was found for the behavior of bacterial MscL that were inserted into pure lipid membranes (Kung, 2005). The stimulus–response relation is very steep, indicating that this MSC belong to the most sensitive MSCs discovered to date. This is also reflected by the stimulus–response relation of the macroscopic receptor current that is also very steep with a power function of between 3 and 5 in a Stevens' power law concept. In addition, the receptor current amplitude is greater in the rapidly adapting neuron as compared to that in the slowly. It is obvious that the fact that the organ is specialized for mechanosensory detection is reflected in the properties and densities of the MSCs present in the neurons. This points toward a real challenge for the future, namely to define the molecular nature of the crayfish MSC represented in the slowly and rapidly adapting neurons.

The voltage-gated Na^+ and K^+ channels are important players in the generation of the action potentials and thus the final impulse response of the two neurons. It has been pointed out in this chapter that differences in adaptive properties between the two receptor neurons can be partly explained by the viscoelastic properties of the receptor muscles and by possible differences in MSC setup and by activation of Ca^{2+}-activated K^+ currents. However, since the same adaptive properties seem to be present also with electrical stimulation (Rydqvist and Purali, 1993; Fig. 1), the voltage-gated ion channels must also be involved in the adaptive characteristics of the two receptors. Several factors could contribute.

First, the type of Na^+ channels present in the two types of neurons. This is supported by the observation that two types of Na^+ channels seem to be present in the neurons. The two Na^+ channels, in all probability, seem to have different kinetic properties. It is shown in model experiments (Rydqvist *et al*., 2003) that small differences in activation and inactivation of the Na^+ channels can have profound effects on the firing properties.

Second, the distribution of Na^+ channels is different in the two neurons. This is supported by observations of Na^+ currents in neurons with axons cut at different positions (Lin and Rydqvist, 1999). The result indicates that in the rapidly adapting neuron, the Na^+ channels are present in the axon only, whereas in the slowly adapting neuron Na^+ channels are present in both axon and soma. This would affect the initiation site of the action potential. In the future, this must be further investigated and determined using histological as well as patch clamp techniques.

Third, the K^+ channels are not the same in the rapidly and slowly adapting neurons. Four types of K^+ channels have, so far, been tentatively defined in the slowly adapting neuron: three types of voltage-gated K^+ channels and one Ca^{2+}-activated K^+ channel. It is not known if the same type of K^+ channels are present in the rapidly adapting neuron or if the relative proportion of the channels is different. Purali and Rydqvist (1992) demonstrated, using pharmacological dissection, that the type of voltage-gated K^+ channels is not the same in the two neurons. Also, the action potentials in the two neurons are different, the one in the rapidly adapting neuron having a much faster repolarization (Purali and Rydqvist, 1998). The explanation of these differences has to be further studied in the future.

Fourth, the spatial distribution of K^+ channels in the two neurons is unknown. In particular, the relation between the Na^+ channel densities, MSC densities, and K^+ channels densities are important. K^+ channels in the soma could act as a current sink for the current generated by the MSCs in the dendrites. This would affect the generation of action potentials, particularly in relation to where the Na^+ channels are located. To solve these

problems we have to use a combination of morphological, electrophysiological, and model studies of this preparation.

Investigations of this relatively simple invertebrate receptor are of fundamental importance for the general understanding of generation of signals in sensory receptors. The results will also give a better insight into the proprioceptive contribution to motor control and how it is possible to modify the function of such receptors, that is, the muscle spindle and tendon organs. In particular, it should be of interest to apply the results to clinically useful therapies (in different pathological conditions where the reflexes involving the muscle receptors are affected). Theoretically, this is possible through the muscle receptors since many motor conditions are generated through increased activity in reflex arcs dependent on mechanosensory organs. One such possibility should be to selectively block the MSCs. At present this has not been generally possible and until the molecular details of the invertebrate and vertebrate MSCs are known, this will be difficult. In this context, it should also be noted that MSCs are probably not a homogenous entity. Several molecular constructions can probably be involved in this sensory modality. However, looking at the rate of development of molecular techniques, the determination of the MSCs in different species including man should not be too far ahead.

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