Receptor Potentials and Electrical Properties of Nonspiking Stretch-Receptive Neurons in the Sand Crab *Emerita analoga* (Anomura, Hippidae)

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**Paul, Dorothy H. and Jan Bruner.** Receptor potentials and electrical properties of nonspiking stretch-receptive neurons in the sand crab *Emerita analoga* (Anomura, Hippidae). *J. Neurophysiol.* 81: 2493–2500, 1999. Four nonspiking, monopolar neurons with central somata and large peripheral dendrites constitute the sole innervation of the telson-uropod elastic strand stretch receptor in *Emerita analoga*. We characterized their responses to stretch and current injection, using two-electrode current clamp, in intact cells and in two types of isolated peripheral dendritic segments, one that included and one that excluded the dendritic terminals (mechanosensory membrane). The membrane potentials of intact cells at rest (mean ± SD: −57 ± 4.4 mV, n = 30), recorded in peripheral or neuropil processes, are similar to the membrane potentials of isolated dendritic segments and always less negative than membrane potentials of motoneurons and interneurons recorded in the same preparations. Ion substitution experiments indicate that the membrane potential is influenced strongly by Na⁺ conductance, probably localized in the mechanotransducing terminals within the elastic strand. The form of the receptor potential in response to ramp-hold-release stretch remains the same as stretch amplitude is varied and is not dependent on initial membrane potential (−70 to −30 mV) or recording site: initial depolarization (slope follows ramp of applied stretch), terminated by rapid, partial repolarization to a plateau (delayed depolarization) that is intermediate between the peak depolarization and the initial potential and sustained for the duration of the stretch. Responses to depolarizing current pulses are similar to stretch-evoked receptor potentials, except for small amplitude stimuli; an initial peak occurs only in response to stretch and probably reflects elastic recoil of the extracellular matrix surrounding the dendritic terminals. The rapid, partial repolarization depends on holding potential and is abolished by 4-aminopyridine (4-AP; 10 mM), implicating a fast-activating, fast-inactivating K⁺ conductance; TEA (60 mM) abolishes the remaining slow repolarization to the plateau. In intact cells, but not dendritic segments, regenerative depolarizations can arise in response to stretch or depolarizing current pulses; they are reduced by CdCl₂ (10 μM) in the saline containing TEA and 4-AP and probably reflect current spread from Ca²⁺ influx at presynaptic terminals in the ganglion. We found no evidence for other voltage-activated conductances. Unlike morphologically similar “nonspiking” thoracic receptors of other species, *E. analoga*’s nonspiking neurons are electrically compact and do not boost the analogue afferent signal by voltage-activated inward currents. The most prominent (only?) voltage-activated extra-ganglionic conductances are for potassium; by reducing the slope of the stretch-plateau depolarization curve, they extend each neuron’s functional range to the full range of sensitivity of the receptor.

INTRODUCTION

The capacity of a neuron to convey information generally is assumed to be related to the nature of the information. The ubiquity of coding by frequency and temporal patterning of action potentials (Perkel 1970) made the discovery of nonimpulsive, graded signals in one class of crustacean mechanosensory neurons (Ripley et al. 1968) unexpected. Analogue signals in nonspiking local interneurons, as well as graded transmission between neurons capable of generating action potentials, are now well documented in regionally localized networks where distances are short (Burrows and Laurent 1989; Burrows and Siegler 1976; Graubard 1978; Graubard et al. 1980; Laurent 1993; Mendelson 1971; Paul and Mulloney 1985a,b; Wolf and Büschges 1995). The “nonspiking” stretch-receptive neurons (NSRs) in decapod crustaceans, on the other hand, must transmit signals from the periphery, where their mechanotransducing channels are located, to their output synapses in the CNS. All are unusual for arthropod sensory neurons in having monopolar, centrally located somata. They all monitor position and movement of the basal joint of the limb (or the equivalent in the case of the oval organ) (Pasztor and Bush 1982), and although some are truly nonspiking, incapable of generating action potentials (Cannone 1987; Paul 1972; this study), others employ graded or constant amplitude action potentials in addition to electrotonic spread of the receptor potential depolarizations (Bush and Pasztor 1983; Cannone and Nijland 1989; Lin and Llinas 1993; Lowe et al. 1978; Mirolli 1981; Pasztor and Bush 1982; Roberts and Bush 1971). The analogue signaling by these proprioceptors is made more enigmatic by the occurrence of morphologically similar (except for the smaller diameter peripheral process) spiking stretch receptors in comparable positions, mediating apparently similar reflexes, at the homologous joints in other species (Miyani and Neil 1986; Paul and Wilson 1994). Nevertheless, NSRs have evolved repeatedly because they occur variably in different body segments in at least half a dozen families, which suggests recurrent selection for analogue signaling in different species.

The receptor potentials and physiological properties of the thoracic members of this small group of mechanosensory neurons have been investigated in some detail (Blight and Llinas 1980; Bush 1976; Bush and Pasztor 1983; Cannone 1987; Cannone and Nijland 1989; Lin and Llinas 1993; Lowe et al. 1978; Mirolli 1979, 1981, 1983; Pasztor and Bush 1982; Ripley et al. 1968; Wildman and Cannone 1996), whereas the more caudal NSRs, associated with abdominal appendages (Heitler 1982; Paul 1972), are less well characterized. The absence of a receptor muscle and...
the more stereotyped movements of the associated appendages (swimmerets in crayfish, uropods in hippid sand crabs) should make the abdominal NSRs more accessible to functional analysis of analogue signaling in this morphologically distinctive type of sensory neuron.

We used standard intracellular microelectrode techniques and pharmacological methods to characterize the responses to stretch and to current injection of the four NSRs innervating the telson-uropod stretch receptor in the sand crab *Emerita analoga*. The somata of these monopolar neurons are in the terminal ganglion of the CNS (Paul 1972) (Fig. 1) and their large (20–40 μm, depending on size of animal) peripheral dendrites extend ≈2 mm to an elastic strand in which their mechanosensitive termini are embedded (Wilson and Paul 1990). *E. analoga*’s peculiar mode of swimming (with the uropods) and their nonspiking telson-uropod stretch receptor evolved together (Paul and Wilson 1994), suggesting a direct functional correlation that, if understood, might provide insight into the significance of analogue signaling in NSRs in general. Preliminary reports of some of this work have been published (Paul and Bruner 1991, 1993).

**METHODS**

*E. analoga* were collected from beaches of Monterey Bay, CA, and held in a recycling sea water facility at the University of Victoria. Large specimens (carapace length 2.7–3 cm) provided the receptors used in the experiments came from smaller animals (carapace length ≥1.6 cm).

The crabs were anesthetized by chilling and perfused with cold saline, to minimize clotting of blood cells around the receptors, before removing the caudal abdominal nerve cord with, usually, both telson-uropod stretch receptors attached to the terminal ganglion (G6) of the ventral nerve cord. By keeping sufficient tissue on either side of the region of the elastic strand occupied by the NSRs’ dendritic termini (Figs. 1, C–E, and 2A), the latter were not damaged or compressed when pinning the tissue on silicone elastomer (Sylgard) resin (Dow Corning) in the experimental chamber.

The strand’s attachment to the telson is between muscles and had to be cut, but a cotton thread ligature placed around this end of the elastic strand (the NSR recorded from could not be determined unless a dye-filled electrode was used); I and Vm, in a distal dendrite, for current-passing and voltage recording, respectively. l1 and l2, ligatures for current-clamp experiments on distal segments (DS; l1) and distal segments without strand (DSWS; both l1 and l2) of an individual NSR. B, direction of pull applied to elastic strand. Inset: side view of a swimming *E. analoga* with uropods (u) extended as at end of return stroke, when receptor strand is maximally stretched (Paul 1972). E: receptor potentials in NSR II elicited by different amplitudes of ramp stretch-hold-release stimuli (bottom). Inset: simultaneous receptor potentials in NSR III (RP = −60 mV), recorded −400 μm outside G6, and NSR II (RP = −56 mV), recorded just inside G6. The high-frequency oscillations during the plateau phase of the receptor potential were due to small, high-frequency lateral oscillations of the forceps, not detectable by the linear variable differential transformer (LVDT), which added short, fast stretches to the constant (hold) phase of the applied stretch. Cells were subsequently injected with Lucifer yellow and Texas Red, respectively. Inset scale bars: 10 mV, 10 ms.

**FIG. 2.** A: diagram of receptor and G6 (ventral view, anterior toward top) showing placements of microelectrodes for the different experiments: Vs, at the edge of G6, for recording receptor potentials in response to stretch of the elastic strand (the NSR recorded from could not be determined unless a dye-filled electrode was used); I and Vm, in a distal dendrite, for current-clamp experiments on distal segments (DSWS; l1) and distal segments without strand (DSWS; both l1 and l2) of an individual NSR. Scale bars: 10 mV, 10 ms.
strand (lig. in Fig. 1E) facilitated manipulation of this delicate structure and provided a sturdy site for attaching forceps to apply stretch stimuli. Minimum and maximum lengths of the portion of the elastic strand containing the NSRs’ terminals measured in situ, with the uropods in rest and fully extended positions, were used to gauge the appropriate range of stretch amplitudes to use after the dissection.

For intracellular recording experiments on portions of the NSRs’ peripheral dendrites, we placed additional ligatures. One ligature around the proximal end of the receptor nerve isolated the distal segments (DS) of all four peripheral dendrites from the central portion of the cells (l1 in Fig. 2A). This nerve branches from the large nerve leaving G6 about half-way between G6 and the elastic receptor strand. Note that DS include the mechanotransducing membrane of the dendritic termini, which are embedded in the elastic strand. Another ligature around an individual dendrite just outside the elastic strand (l2 in Fig. 2A) excluded the stretch-activated channels, generating a distal segment without strand (DSWS) 425–500 μm in length.

Controlled stretches of the receptor were made via fine forceps that gripped the elastic strand close to or on the ligature posterior to the NSR terminations (see preceding text; Fig. 1E). The forceps were glued to one end of the core of a DC-operated linear variable differential transformer (LVDT; Schaevitz, Pennsauken, NJ), which gave a record of the stretch stimuli delivered; the other end of the LVDT core was fixed to the center of an 8-Ω speaker cone. Ramp stretch-hold-release commands to drive the actuator for the speaker were shaped either by a Phillips PMS153 function generator or by one of the analog out-channels in the Clampex program (Pclamp software, Axon Instruments, Foster City, CA) programmed to generate ramp stretch-hold-release sequences.

Microelectrodes were pulled from 1.2 mm, standard-wall, glass capillary (Clare Electromedical Instruments) and filled with 2.7 M KCl. Resistances of electrodes for two-electrode current clamp were between 6 and 10 MΩ. Electrodes for recording in bridge mode from sites close to or inside the ganglion had higher resistance (15–35 MΩ). In the latter experiments, dye-filled electrodes sometimes were used to reveal the identity (by serial order of termination along the elastic strand) and central morphology of the NSR. Injected dyes move preferentially toward the periphery in these neurons, so very small amounts suffice for identification, but larger amounts are needed to make visible their central structure (Fig. 1). Either Lucifer yellow (3–5% in 0.1 M LiCl) or Texas Red (sulforhodamine 101 acid chloride; 3% in 0.1 M KCl) was injected by 1-Hz, 4- to 10-nA, 500-ms, hyperpolarizing current pulses.

Ganglia were fixed in 10% formaldehyde in saline for 1–2 h or for 20 min followed by 1-h overnight in 10% methyl formcel in methanol, dehydrated (or directly into 100% ETOH from methyl formcel), and cleared and mounted in methylsalicylate in a depression slide for viewing and photographing through a Leitz Aristoplan epifluorescence microscope.

Signals were recorded via an Axoclamp 2A amplifier (Axon Instruments) and displayed on an oscilloscope. Initially, we recorded data photographically on film or paper, using a Nikon-Kohden camera mounted on a Textronix 565 oscilloscope; these records were later digitized or scanned for plotting and preparation of figures. In later experiments, we used PClamp software (Clampex, Clampfit programs; Axon Instruments), stored data on computer, and used origin technical graphics and analysis software (MicroCal Software, Northampton, MA) for preparing graphs.

Aerated solutions were superfused continuously through the experimental chamber (full volume 2 ml) at a rate of 2.8–3.0 ml/min. The temperature at the inflow was kept at 14 ± 0.5°C by a Peltier cooling device. During pharmacological experiments, the volume was kept minimum (−0.8 ml) for rapid exchange of solution. A minimum of 20 min of superfusion with normal saline separated the delivery of each test solution, which was superfused through the chamber for 6–10 min while the test stimulations were made. Emerita salina (ES) contains (in mM) 460 NaCl, 12.7 KCl, 13.7 CaCl2, 10 MgCl2, and 14 Na2SO4, buffered with N-[2-hydroxyethyl] piperazine-N’-[2-ethylsulfonic acid] (HEPES) at pH 7.5. Glucose (0.9 g/l) sometimes was added to the saline, although preparations in saline without glucose showed no signs of deterioration for many hours. Salines of different ionic compositions were prepared from 1 M stock solutions according to the protocol of Gola and Selverston (1981). Solutions for pharmacological experiments were made up fresh the day of the experiment; concentrations to produce maximum, reversible effects were used in the experiments reported here. NaCl was reduced in saline containing tetraethylammonium chloride (TEA+, final concentration 60 mM) by equivalent millimoles; 4-aminopyridine (4-AP; final concentration 10 mM) was dissolved in ES shortly before use. Veratridine (100 μg) was dissolved in 10 μl DMSO and 10 μl 100% ETOH, then diluted to 10−5 M in ES. A 3 × 10−3 M stock solution of tetrodotoxin (TTX) was diluted in ES before use. The efficacy of the veratridine and TTX solutions in blocking action potentials was verified by recording extracellularly from a motor nerve or connective and observing reversible blocking of tonic activity. All chemicals were from Sigma.

RESULTS

The four nonspiking stretch receptors (NSRs I–IV) of E. analoga’s telson-urodop stretch receptor have monopolar somata located medial to the principle neurites, two in a relatively posterior position and two more central, within the last abdominal ganglion (Fig. 1, A and B) (Paul and Wilson 1994). The principal neurite of each neuron gives off short branches into medial and lateral neuropil before expanding to become the large dendrite that exits the ganglion. In the periphery, the dendrites terminate sequentially (I–IV, anterior to posterior) in an elastic strand strung between the inner dorsal telson and the medial-ventral rim of the uropod propodite (Figs. 1, C–E, and 2A) (Paul 1972). We studied the membrane responses of the four NSRs to stretch and to intracellular current injection in three types of preparation: intact neurons; the distal one-third to one-half of the peripheral dendrite (DS), isolated from the proximal portion of the dendrite and soma by a tight ligature around the receptor nerve (l1 in Fig. 2A); and similar distal segments that were isolated from their stretch-sensitive terminals in the elastic strand (DSWS) by a second ligature (l2 in Fig. 2A).

NSRs at rest

When the elastic strand is relaxed, the membrane potentials of the NSRs recorded in or near the ganglion are typically −55 to −64 mV: [−57.8 ± 4.4 (SD) mV; n = 30] and usually 3 to −8 mV lower (depolarized) than those of motoneurons and most interneurons (neuropil recordings) in the same preparations. The membrane potentials recorded in the distal segments were similar: NSR I, −63.4 ± 3.4 mV (n = 5); NSR II, −59.9 ± 4 mV (n = 8); NSR III, −58.6 ± 8.8 mV (n = 14); NSR IV, −62 ± 3.3 mV (n = 4); and no different when the distal segments were separated by ligature from the neurons’ proximal portions (−58.9 ± 4.9 m; n = 9).

We did not thoroughly investigate the ionic dependence of the NSRs’ membrane potential, but three ion-substitution experiments produced similar results and revealed that this potential is influenced strongly by Na+ conductance, probably because of their mechanotransducing terminals. In one of these experiments, for example, substituting sucrose for Na+ increased the membrane potential from −58 to −76 mV within minutes, whereas Li+ in place of Na+ decreased it somewhat.
(to −53 mV). Increasing Ca\textsuperscript{2+} fourfold had little effect, but the membrane potential in Ca\textsuperscript{2+}-free saline was lowered to −54 mV when Na\textsuperscript{+} replaced Ca\textsuperscript{2+} and to −49 mV when Ba\textsuperscript{2+} replaced Ca\textsuperscript{2+}. In every case except after Ba\textsuperscript{2+} in place of Ca\textsuperscript{2+} in the saline, the resting potential quickly returned to the control value of −58 mV; recovery after exposure to Ba\textsuperscript{2+} was slower and incomplete.

**Receptor potential**

By impaling an NSR’s dendrite close to or inside the ganglion, the elastic strand can be stretched without dislodging the microelectrode and the resultant receptor potential recorded intracellularly (Fig. 2). When the tissue had been pinned to allow stable recordings from more distal sites, close to the branch point of the receptor nerve, the receptor potentials recorded in response to small to moderate stretch were similar in form to those recorded routinely from penetrations close to or inside the ganglion. Figure 2B, inset, shows the receptor potentials recorded simultaneously, with dye-filled microelectrodes, from NSR II, penetrated just inside the ganglion, and NSR III, penetrated well outside of the ganglion, close to where the receptor nerve branches from the main nerve. The rapid, synchronous oscillations of their membrane potentials during the plateau phase were the result of lateral vibrations of the forceps gripping the elastic strand which, at certain positions of the LVDT, are unavoidable with rapid stretch. (These lateral movements are visually apparent at ×20 magnification, but not detected by the LVDT because they are perpendicular to its core.) This dual recording is interesting for both the similarity in form and amplitude of the receptor potentials recorded in the ganglion (NSR II) and >500 μm outside the ganglion (NSR III) and for the illustration of how precisely small, rapid signals are transmitted by these neurons.

The general form of the receptor potential remains the same as stretch amplitude is varied: rapid depolarization (of a few millivolts from initial potential, for the smallest stretches, to depolarizations to about −10 mV for the largest stretches), which is terminated abruptly by a rapid, partial repolarizing swing of the membrane potential that merges with a stable plateau depolarization (called delayed depolarization) intermediate between the peak depolarization and the initial potential (Fig. 2B). The peak and subsequent delayed depolarizations increase with increasing stretch amplitude, but neither ever reaches 0 mV (Fig. 3). For moderate to large stretches, the rapid repolarization phase (which ends the initial “peak’) begins before the end of the ramp (Figs. 2B and 3C, inset), but note that even the smallest stretches produce a small peak (Figs. 2B and 3A). No peak occurs with low-velocity stretches of any amplitude, rather the receptor potential follows the stimulus wave form.

Neither the form nor amplitude of the receptor potential is influenced strongly by resting potential. Low membrane potentials (less than −50 mV and as low as −30 mV), whether due to poor penetration, tonic stretch of the elastic strand, or pharmacological treatment, do not significantly modify the form of the receptor potentials generated in response to stretch. The “negative dynamic” mimics the ramp relaxation phase of the stretch stimuli, being an approximate mirror image of the depolarizing phase, except that a substantial “negative peak” (afterhyperpolarization) occurs only when the elastic strand is initially under slight tension; this implicates elastic properties of the strand rather than voltage-sensitive conductances as its cause.

Regardless of the membrane potential at rest in ion-substi-
tuted saline (see preceding section), the amplitudes of both peak and delayed depolarization of receptor potentials were unaffected when Li\(^+\) replaced Na\(^+\) in the saline but were reduced to one-third of control amplitudes when Na\(^+\) was replaced by sucrose. Na\(^+\) in place of Ca\(^{2+}\) increased both peak and delayed phase ~150%. The peak was unchanged but the delayed depolarization dramatically increased (>300%) when Ba\(^{2+}\) replaced Ca\(^{2+}\) in the saline.

**Electrical properties of NSRs**

In both whole cell and isolated distal segments of NSRs, depolarization by current injection through the second intracellular electrode produces an initial, fast depolarization, which, when sufficiently large, is terminated by a fast, partial repolarization that, like the partial repolarization after stretch-induced initial peaks, merges with a second, delayed phase of depolarization to achieve a steady plateau (Fig. 4). Steady-state current-voltage relations (measured at 40 ms, by which time maximum responses had been reached) are linear for hyperpolarizing current pulses but show rectification for depolarizations, starting ~15 mV depolarized (at about ~40 to ~45 mV). Input resistances measured in a distal segment were initially between 0.6 and 1.5 M\(\Omega\) in intact cells and 1–2 M\(\Omega\) in ligated distal segments and usually increased over time to 2–3 M\(\Omega\).

The fast repolarization depends on the holding potential. Figure 5A shows responses of an isolated distal segment of NSR III to two series of depolarizing pulses applied from different holding potentials, ~60 mV (the resting potential in this cell) and ~75 mV. The peak depolarizations reached the same absolute level (~10 mV) for the largest current pulse in both series (Fig. 5A). For all NSRs studied, shifting the holding potential toward progressively less negative values reduced, and then eliminated, the repolarizing phase (Fig. 5B). A hyperpolarization immediately preceding a depolarizing current pulse from a holding potential of ~60 mV increased the repolarization after the peak (Fig. 5C).

**Ionic dependence of the NSRs’ responses**

Potassium-channel blockers have pronounced effects on whole cell responses. The partial repolarizing swing of receptor potentials in response to stretch of the elastic strand, recorded proximally at the edge of the ganglion, is abolished (Fig. 6, A, C, and DJ). The effect is similar on responses to injected depolarizing current, recorded in the distal dendrite (Fig. 6B2). [The small dip in the rising phase of the receptor potential (Fig. 6, A and C) reflects elastic adjustment in the receptor strand after the end of the ramp stretch.] The resultant increase in both delayed depolarizations (produced by stretch or current injection), can become regenerative and outlast the stimulus (Fig. 6B). These delayed depolarizations are reduced by CdCl\(_2\) (10 \(\mu\)M) in the saline containing 60 mM TEA\(^+\) (Fig. 6, A and C). This suggests that this depolarization, which can overshoot 0 mV by a few millivolts and become regenerative (Fig. 6B), results from a voltage-activated Ca\(^{2+}\) conductance added to the stimulus (stretch or injected current)-induced depolarization. In normal conditions, it is at least partially antagonized, probably by an outward current during the delayed phase of the response. CdCl\(_2\) (10 \(\mu\)M) in normal saline produces a small, reversible increase in plateau amplitude (not shown), implicating a small contribution to the normal delayed response from a Ca\(^{2+}\)-activated K\(^+\) conductance.

Spontaneous fluctuations and oscillations of membrane potentials are common in relaxed, unstimulated NSRs in TEA\(^+\) saline and are particularly prevalent immediately after the receptor has been stretched repeatedly; at times they resemble synaptic potentials recurring singly or in short bursts (Fig. 6D2), implicating current spread from synaptic terminals as their source.

The effects of K\(^+\)-channel blockers on dendritic segments and on whole cells differ. The fast repolarization phase in both whole cells and dendritic segments is eliminated by 4-AP (10 mM), the initial depolarization being instead reversed by a slower, partial repolarization to the plateau level (Figs. 6A, trace 2, and 7A). In dendritic segments, TEA\(^+\) (in addition to 4-AP) abolishes the repolarization phase and thereby eliminates the broad transient, so the response mimics the square

![FIG. 4. NSR membrane responses (right) to hyperpolarizing and depolarizing current pulses (40 ms) of increasing amplitude. With the larger depolarizing current pulses, the fast depolarization is reversed partially by fast repolarization, followed by a 2nd, slower phase of depolarization to a plateau. Current-voltage graphs (left): ordinate, membrane potential (mV) at end of 40-ms pulses; abscissa, injected current (nA). A: NSR III (whole cell) responses (right) and I-V curve for a different NSR III (left). B: isolated distal segment of NSR III (DSWS)—note higher input resistance than in A. C: NSR IV (DS).](Image 324x347 to 564x731)
waveform of the current pulse (Fig. 7B1). However, dendritic segments (DS and DSWS) do not develop the regenerative depolarizations observed in whole cells when K⁺-channel blockers are present. This is evidence that these depolarizations arise in proximal, presumably presynaptic terminal, portions of these neurons, which the morphology (Fig. 1, A and B) suggests are electrically close to the large, peripheral dendrite.

We found no evidence for voltage-activated sodium inward current in *E. analoga*’s NSRs. The amplitude of the depolarization reached by the peak increases progressively with increasing amplitudes of stretch or injected depolarizing current. There is no evidence of an inflection or step increase in the rising phase of the response, such as would be expected were there a voltage-activated sodium inward current. The absence of voltage-activated Na⁺ channels also is indicated by the facts that neither the slope nor amplitude of the peak depolarization in response to stretch is dependent on initial membrane potential (Fig. 6A, traces 1 and 4) and the slope of the peak potential in response to depolarizing current pulses is not modified by shifting the membrane potential to different values (Fig. 5). Finally, bathing the preparation in TTX (10⁻⁷ M) and TEA (10⁻⁵ M) had no obvious effect on the responses to current pulses that depolarized the NSR up to 0 mV from a holding potential of −65 mV (data not shown).

**Discussion**

The results of this study reinforce the impression from functional and ultrastructural data (Paul 1972; Wilson and Paul 1990; Paul, unpublished observations) that the four NSRs,

![Image](https://via.placeholder.com/150)

**FIG. 5.** A and B: fast repolarization phase depends on the holding potential. A: NSR III (DSWS) responses to a series of depolarizing pulses applied from 2 holding potentials, −60 mV (the resting potential) and −75 mV. (bottom) indicate that initial depolarization reached the same level (equals −10 mV). B: shifting the holding potential toward less negative values reduces, then eliminates, the fast repolarizing phase (NSR II, DS). C: hyperpolarizing current pulse preceding a depolarizing current pulse increases the amplitude of the fast, transient repolarization, NSR IV (DS).

which constitute the entire innervation of the elastic strand of *E. analoga*’s telson-uropod stretch receptor, are not differentiated from each other and that each NSR responds over the full range of the receptor. The responses to stretch and depolarizing current pulses are very similar to each other, except for small amplitude stimuli: the initial peak depolarization preceding the sustained plateau occurs only in response to stretch and not to current injection (Figs. 2B and 4A). The partial repolarization during stretches of such small amplitudes could be due to one or more of the following: activation of voltage-dependent outward conductance, adaptation of the mechanosensory channels, and relaxation of the extracellular matrix in the elastic strand.

Our current-clamp data provide no evidence that the first mechanism could be involved. In fact, the depolarization of the membrane at the recording site by only a few millivolts from rest potential apparently did not activate the outward conductances.
The ability of the NSRs to sustain plateau depolarizations for minutes militates against adaptation of mechanoelectric transducing channels, which leaves decline in the mechanical stimulus received by the dendritic termini, due to compliance of the elastic strand, as probable cause of these partial repolarizations. The elastic properties of the receptor strand most likely also underlie the small peak that persists in receptor potentials elicited in the presence of potassium-channel blockers (Fig. 6). A and B1: same scale (shown in A2).

The dye-fills confirmed that the terminations of the four NSRs occupy approximately similar-sized, nonoverlapping regions in the elastic strand. Wilson and Paul (1990) detailed how, inside the elastic strand, the large branches of each NSR’s dendrite give rise to a very large number of short, uniform (1 × 10 µm) dendritic termini, all parallel to the long axis of the elastic strand and surrounded by a distinctive extracellular matrix. These termini undergo differential compression of their distal portion and expansion of their proximal portion when the strand is stretched, and Wilson and Paul (1990) proposed that it is the expansion of the proximal portions that opens stretch-activated channels. The ultrastructural appearance of the dendritic terminations and surrounding extracellular matrix is similar for other NSRs (reviewed in Wilson and Paul 1990); presumably similar events in the peripheral terminals of all members of this morphological type of neuron link the mechanical stimulus to the mechanoelectric transducers, and, as modeled by Berger and Bush (1979), account for the gross similarity in form of their receptor potentials.

Our data indicate that, in addition to their mechanotransducing channels, E. analoga’s NSRs have a remarkably simple complement of voltage-activated conductances. A fast-activating, fast-inactivating outward conductance, which is blocked by 4-AP and resembles $K_A$-conductances in a wide spectrum of other cells (Rogawski 1985) including nonspiking interneurons (Laurent 1993), mediates the fast, partial repolarization that, except for small amplitude stimuli, cuts short the depolarizing swing of the membrane potential in response to stretch or depolarizing current. Its effect is to narrow the peak, i.e., accelerate the slower repolarization due to elastic recoil of the extracellular matrix in the receptor strand. This restrains the depolarization until a slower, TEA$^+$-sensitive conductance develops that maintains the stable, delayed depolarization phase in the face of the continuing depolarizing drive due to the stretch or current injection. We could find no indication of any other voltage-activated conductance in the extraganglionic portion of E. analoga’s NSRs.

The sometimes overshooting, regenerative responses that develop in the presence of TEA$^+$ and are blocked by cadmium occur only in intact cells, not in dendritic segments that exclude synapses-bearing neuropil processes. This suggests that they result from the spread of current generated by Ca$^{2+}$ influx through voltage-sensitive Ca$^{2+}$ channels at the presynaptic terminals (Blight and Llinas 1980). CdCl$_2$ (10 µM) in saline produced a modest reduction in amplitude of large stretch-induced plateau depolarizations (not shown). A calcium-activated, outward (K$^+$) conductance also may be present in the neuropil segments.

Unlike all of the thoracic NSRs that boost their initial transients by a voltage-activated Na$^+$ conductance (Bush and Pasztor 1983; Cannone and Nijland 1989; Lowe et al. 1978; Mirolli 1979, 1981, 1983; Pasztor and Bush 1982), with the possible exception of the D neuron (Cannone 1987), E. analoga’s NSRs appear to have no voltage-activated inward-conducting channels in their peripheral dendrite. This correlates with the relatively short distance (2 mm maximum in large individuals of 3 cm carapace length) over which the receptor potentials must spread to reach the output synapses in the ganglion compared with several centimeters for some of the thoracic NSRs. For the latter, it has been suggested that the active component of their responses is needed as much for compensating capacitative slowing as for boosting amplitude of decrementing, analog signals that must spread over long distances (Mirolli 1983).

Although we have been unable to maintain stable microelectrode penetrations close to the periphery while stretching the elastic strand to record the receptor potential close to its source, it is clear that neither attenuation nor distortion is substantial over the full distance that the receptor potential of E. analoga’s NSRs must spread. Small stretches applied to a taught (unstretched) elastic strand produce ~1 mV receptor potentials recorded in the ganglion, which effect transmitter release (Paul 1989a); also, at these central recording sites, rapidly varying mechanical stimuli are followed with little distortion by fluctuations in membrane potential. Without outward conductances to restrain the amplitude of depolarizations reaching the ganglion in NSRs as short as E. analoga’s, saturation at the output synapses could occur with even small stretch stimuli. By lowering the slope of the stretch-depolarization curve, the voltage-sensitive K$^+$ conductances extend these neurons’ functional range to include the full range of sensitivity of their peripheral mechanotransducing termini, which is equal to that of the receptor as a whole (Wilson and Paul 1990); the latter role of K$^+$ conductance has been identified in other sensory cells (Pepose and Lisman 1978). The superior ability of graded...
potentials to transmit information (de Ruyter and Laughlin 1996) may explain their common occurrence in sensory systems where the amount/rate of information transfer needs to be high. This exigency seems unlikely for proprioceptors at basal limb joints (not all of which are nonspiking) and suggests that other explanations for analog signals in some members of the small group of crustacean stretch receptors with central somata should be considered (Paul 1989b).

These comparative data suggest that the nonspiking members of this morphological class of sensory neuron are primarily nonspiking (rather than having lost voltage-activated depolarizing conductances) (Mirolli 1981) and secondly have added some inward conductance when needed to transmit signals over longer distances as in the thorax. The combination of analog and spiking transmission was exploited for further enrichment of signaling in ways appropriate for each situation (Cannone and Nijland 1989; Pasztor and Bush 1982; Wildman and Cannone 1996).

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