ION CHANNELS, TRANSPORTERS

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Effects of intracellular pH and Ca^{2+} on the activity of stretch-sensitive cation channels in leech neurons

Abstract The effects of intracellular pH and calcium on the activity of the leech mechanosensitive cation channels have been studied. These channels exhibited two activity modes denoted as spike-like (SL) and multiconductance (MC). In the absence of mechanical stimulation, acidification of the intracellular side of membrane patches from 7.2 to 6.2 reversibly increased the mean channel open time as well as the opening frequency in the SL mode. Channels in MC mode were activated by a pH$_i$ reduction from 7.2 to 6.2, but were inhibited at pH$_i$ 5.5. Unlike MC mode, SL mode was strongly activated by intracellular Ca^{2+}. Fura-2 imaging experiments showed that intracellular calcium was induced to increase by hypotonic cell swelling. The major component of this response did not require extracellular calcium. A component of the swelling-induced calcium response was sensitive to blockers of stretch-sensitive cation channels. The results indicate that the two activity modes of mechanosensitive channels of leech neurons respond differently to changes of intracellular pH and calcium. The sensitivity of the channel to micromolar concentrations of internal free calcium, along with its permeability to this ion, is consistent with a role in the amplification of mechanically induced Ca^{2+} signals in leech neurons.

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Introduction

Single stretch-sensitive ion channels have been identified in membrane patches of leech neurons [30]. These channels are expressed by mechanosensory cells responding to light touch or pressure applied to the skin, as well as by other identified neurons, not involved in sensory mechanotransduction, such as motor neurons. Channel activation was obtained during the application of slow negative pressure to the recording pipette in inside-out configuration or by perfusion with hypotonic solutions in cell-attached configuration [30].

Experiments of channel ion selectivity demonstrated that these stretch-sensitive ion channels admit cations and exhibit a substantial calcium permeability [2]. Their pharmacological features are similar to those of typical mechanogated channels of vertebrate hair cells [12]. They are completely and reversibly blocked by extracellular Gd^{3+}. Amiloride induces a flickering voltage-dependent block and gentamicin produces a complete voltage-dependent block. Both amiloride and gentamicin block the channel at negative membrane potentials [2].

Both cell bodies and growth cones of leech neurons growing in culture express stretch-sensitive cation channels. Although mechanosensitivity, main single-channel conductance, outward rectification, ion selectivity, pharmacological properties, and light and slow voltage sensitivity were shared by all the recorded cation channels, two activity modes differing in kinetics and single-channel subconductances were identified. The first, denoted as spike-like (SL) mode, was mainly displayed in membrane patches excised from freshly desheathed quiescent cell bodies, while the second, called multiconductance (MC) mode, was commonly found in cultured cell bodies and in growth cones [29], where the channels also displayed high mechanosusceptibility [22].

Furthermore, previous findings suggested a role of the leech mechanosensitive channels in cell growth. Addition to the culture medium of gentamicin, a nonspecific blocker of these cation channels, which does not affect voltage-

dependent currents in the leech neurons, increased the neurite extension in culture [2].

Because data concerning the molecular nature of these ion channels are still unavailable, a further characterization of their functional properties was performed to compare them to those of mechanosensitive ion channels belonging to identified structural families.

In this study, we show that leech stretch-sensitive channels show a polymodal activation. They are transiently activated by membrane stretch and are consistently activated by intracellular acidification and intracellular calcium. These features, along with the previously described electrophysiological and pharmacological properties, are consistent with those of transient receptor potential (TRP) channels.

Materials and methods

Adult specimens of *Hirudo medicinalis* L., obtained from a commercial supplier (Ricarimpex, Eysines, France), were used in this study. Animals were anaesthetized with chlorobutanol 0.15%. Segmental ganglia were removed from the central nervous system in standard leech saline [composition in millimolar (mM): NaCl 115, KCl 4, CaCl$_2$ 1.8, Tris-maleate buffer 10, glucose 10, pH 7.4]. Identified anterior pagoda motor neurons and mechanosensory neurons were selected in this study. The capsule enveloping the ganglion was opened with microscissors to allow single cell bodies to stick out for patching. Single cell bodies were also isolated and maintained in culture, according to Fuchs et al. [7]. Cells were plated onto Concanavalin A- (Sigma) coated Petri dishes (Falcon 3001, Becton Dickinson, Le Pont De Claix, France) in Leibowitz-15 medium (Sigma), with 0.6% glucose and 2% fetal bovine serum (Sigma), and maintained at 20–22°C for 3–7 days.

Electrophysiology

The patch-clamp technique [11] in the inside-out configuration was used. Single-channel currents were recorded from naked cell bodies in desheathed ganglia. Patch electrodes, pulled in two stages from 1.5 mm o.d. glass capillary tubes 7087 (Blaubrand, Wertheim, Germany), had resistances of 5–7 MΩ, a "bubble number" [5] of about 4, and were coated with Sigmacote (Sigma). 'Gentle' sealing protocol [13] was routinely applied. In most recordings, we obtained a tight seal just by releasing a positive pressure of about 10 mmHg, without applying negative pressure. Currents were recorded, with a patch-clamp amplifier (RK 300 Biologic, Claix, France), by low pass filtering at 1 kHz with a four-pole Chebyshev filter. Data were displayed on an oscilloscope and stored on the hard disk of a PC for off-line analysis as Axoscope (Axon Instruments, Foster City, CA, USA) files, after AD conversion at 5 kHz with a Digidata 1200 interface (Axon Instruments, Foster City, CA, USA). Analysis was carried out using both pClamp

software (Axon Instruments, Foster City, CA, USA) and software developed for the purpose in Visual Basic. The stretch activation of channels was induced by applying rapid negative pressure stimuli to the membrane patches, using a two-electrovalve system, similar to that described by Hurwitz and Segal [15].

Solutions

In most recordings, symmetrical solutions of pipette and bath contained (in mM): 155 NaCl, 1 MgCl$_2$, 10 HEPES (N-(-2-hydroxyethyl)piperazine-N'-2 ethanesulfonic acid) NaOH, 5 KCl, 1 CaCl$_2$, pH 7.2, and glucose 10. Cell-free patches were tested with solutions of different composition applied by a rapid solution changer. In experiments designed to study the calcium sensitivity of the channels, solutions containing different free calcium concentrations were obtained using 2 mM EGTA (ethyleneglycol-bis[β-aminoethylether]N,N-tetraacetic acid) and following instructions of the MaxC software by C. Patton (Stanford University). The effects of intracellular acidification were determined by perfusing the internal side of inside-out membrane patches with solutions at three values of pH (7.2, 6.2, and 5.5). Solutions at pH of 5.5 were prepared either with 10 mM MES (2-(N-morpholino)ethanesulfonate) or 10 mM acetate buffer.

For calcium imaging experiments, cultured cells were loaded by a 30-min incubation in Leibowitz-15 containing the acetoxy methyl (AM) ester of fura-2 (2 μM) (Molecular Probes Europe, Leiden, The Netherlands) at 20°C in the dark. Cells were then rinsed and allowed to complete hydrolysis of AM ester groups for 30 min. Images were obtained with a fluorescence microscope (Nikon Eclipse 600), using a CFI Fluor 60X, 1.0 NA, water-immersion objective. Images were acquired by a CCD camera (Panasonic, WV-BP514E) and collected using Axon Imaging Workbench 2.2 software (Axon Instruments, CA, USA), by averaging 16 frames (time of exposure 528 ms).

To measure the intracellular free Ca^{2+} levels, fura-2-loaded cells were alternatively excited at 340 and 380 nm while they were imaged at 510 nm at 20°C. After background subtraction, ratio images were obtained by dividing, pixel by pixel, pairs of digitized images at 340 and 380 nm. In each cell, the fluorescence intensity was measured by the mean pixel value, in a region of interest covering most of the cell area. Fluorescence values were converted into ion concentrations according to the equation given by Grynkiewicz et al. [10]:

$$[Ca^{2+}] = K_d \left(\frac{R - R_{min}}{R_{max} - R}\right)\left(\frac{F_{f380}}{F_{b380}}\right),$$

where R is the ratio of the fluorescence intensities measured at 340 and 380 nm, R_{min} is the limiting value of R when all the indicator is in the Ca^{2+}-free form, R_{max} when it is saturated with calcium, K_d is the dissociation constant of

the fura-2-calcium complex, and F_{f380} and F_{b380} are the fluorescence intensities of Ca^{2+}-free and Ca^{2+}-bound fura-2 at 380 nm, respectively. For calibration, Ca^{2+}-saturated or Ca^{2+}-free dye was set by 10 μM ionomycin in the presence of 2.5 mM Ca^{2+} or by 10 mM EGTA in nominally Ca^{2+}-free solution, respectively. All the imaging experiments were performed under continuous perfusion with standard leech saline, at a rate which allowed the bath to be completely exchanged in 10 s. Hypotonic solution was obtained by replacing 30% of the NaCl in the leech saline. The membrane surface area was calculated, assuming a spherical shape.

In channel blocking experiments, gentamicin sulfate (Sigma) was added to the perfusing solution at a final concentration of 200 μM, and gadolinium (III) chloride (Sigma) was applied extracellularly at a concentration of 100 μM.

Data processing and statistical analysis

Transitions between open and closed states were detected using a half-amplitude threshold criterion and a minimum event width of 0.2 ms. The mean patch current was computed by dividing the total ionic charge transported by the duration of analyzed data. The mean frequency of events was obtained by the ratio between the number of opening transitions and the total recorded time. The number of channels in each membrane patch was determined by the maximum number of channels open simultaneously, observed in records longer than 2 min. Only records with a single channel were used to study channel kinetics. Mean channel open and closed times were determined by the arithmetic means of dwell time data.

Mathematical transformations and statistical analysis were performed using Origin (Microcal) and Sigma Stat (Jandel Scientific). Results are expressed as means±SEM (standard error of means) and compared by paired t test, taking as significant P values of less than 5%.

Results

In a previous paper, we reported that large conductance cation channels in the leech were activated by slow application of negative pressure to the patch pipette, in inside-out configuration, as well as by hypotonic swelling, in cell-attached condition [30]. To study the dynamics of the channel response, its activation was induced by applying fast pressure changes whose 20–80% step response times were less than 1 ms. Although channels exhibiting both activity modes gave transient responses to brief and fast mechanical stimuli, SL mode was preferred for characterizing the mechanically induced channel response, mainly due to the absence of spontaneous activity. The response latency was found to vary between a few milliseconds and seconds.

Figure 1a shows an example of prompt channel response during a brief mechanical stretch applied to an inside-out patch containing two cation channels. Figure 1b illustrates how long-lasting stimulations of a patch containing a single mechanosensitive channel in SL mode, at increasing values of negative pressure, induced progressively stronger responses. Channel activation was obtained with pressure steps near −20 mmHg. The channel opening frequency increased with pressure (Fig. 1c), without considerable changes of the mean channel open time. A response adaptation to sustained pressure stimuli was also observed (Fig. 1b).
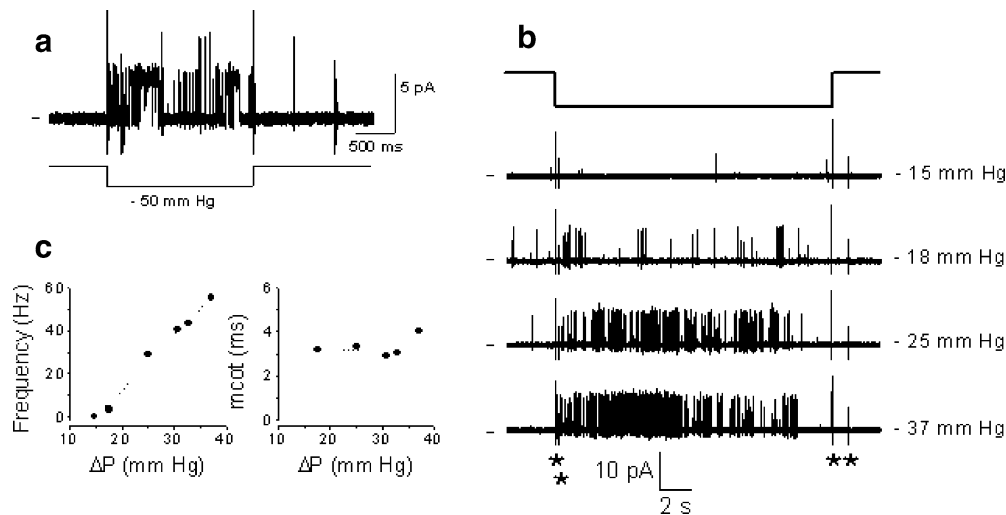
Fig. 1 Activation of leech mechanosensitive cation channels by fast pressure pulses. **a** Prompt activation (latency 8 ms) and deactivation induced by a brief mechanical stimulation, in symmetrical Na^+ solutions, at a membrane potential of +50 mV. **b** Effects of long-lasting stimuli of increasing pressure values, reported on the right of each trace, on a single channel, at a membrane potential of +80 mV.

c Plots of opening frequency and mean channel open time as functions of applied pressure pulse. In all traces, outward currents are displayed as upward deflections. Filtering 1 kHz. The *asterisks* mark the artifacts associated with openings and closures of two solenoid valves controlling the pipette pressure

These cation channels were found to be affected by intracellular acidification. The effect of intracellular pH was studied in excised membrane patches under perfusion, in the absence of pressure stimulation. Figure 2 illustrates the effects of six changes of pH on the mean current (Fig. 2a) and the opening frequency (Fig. 2b) of mechanosensitive channels. Both parameters are reversibly increased by acidosis. Figure 2d shows the mean values of measurements of the mean patch current made at the three different values of pH on 20 patches displaying the SL mode.

The activating effect of low pH_i was owing to an increase in both the opening frequency and the mean channel open time, as shown in Fig. 3a. The single-channel conductance was reduced under perfusion with low pH solutions, as shown in Fig. 3b.

As previously reported, channels in MC mode displayed a higher spontaneous activity than those in SL mode. Mild intracellular acidosis induced a further activation, as in SL mode, but at pH of 5.5, a clear-cut channel inhibition was consistently observed, using both MES and acetate as buffer. In Fig. 4a, an example of the dual effect of low pH on a patch containing four channels in MC mode is shown. The current samples and the corresponding all points histograms in Fig. 4b show that the single-channel conductance is unaffected by pH. The mean values of measurements of the mean patch current, made at the three different pH, on 11 patches displaying the MC mode, are grouped in Fig. 4c.

While the two activity modes were both affected by pH_i, their activity exhibited a clear-cut difference in the sensitivity to changes of intracellular calcium concentration.

The effects of changing the calcium concentration in the solution bathing the intracellular face of mechanosensitive channels were studied in inside-out configuration. Ten out of ten patches displaying the SL mode responded with a consistent and reversible decrease in the mean current when calcium was reduced. A representative example of this response is illustrated in Fig. 5.

In all cases examined ($n=6$), channels in the MC mode were unaffected by changes of intracellular calcium concentration. The selective effect of low calcium on the SL mode can be clearly observed in Fig. 6, where two channels in the same membrane patch exhibit the two activity modes (upper trace). The reduction of the calcium concentration (middle trace) reversibly abolished the SL activity, without modifying the MC activity.

The ability of Ca^{2+} to carry current through leech mechanosensitive cation channels had been previously
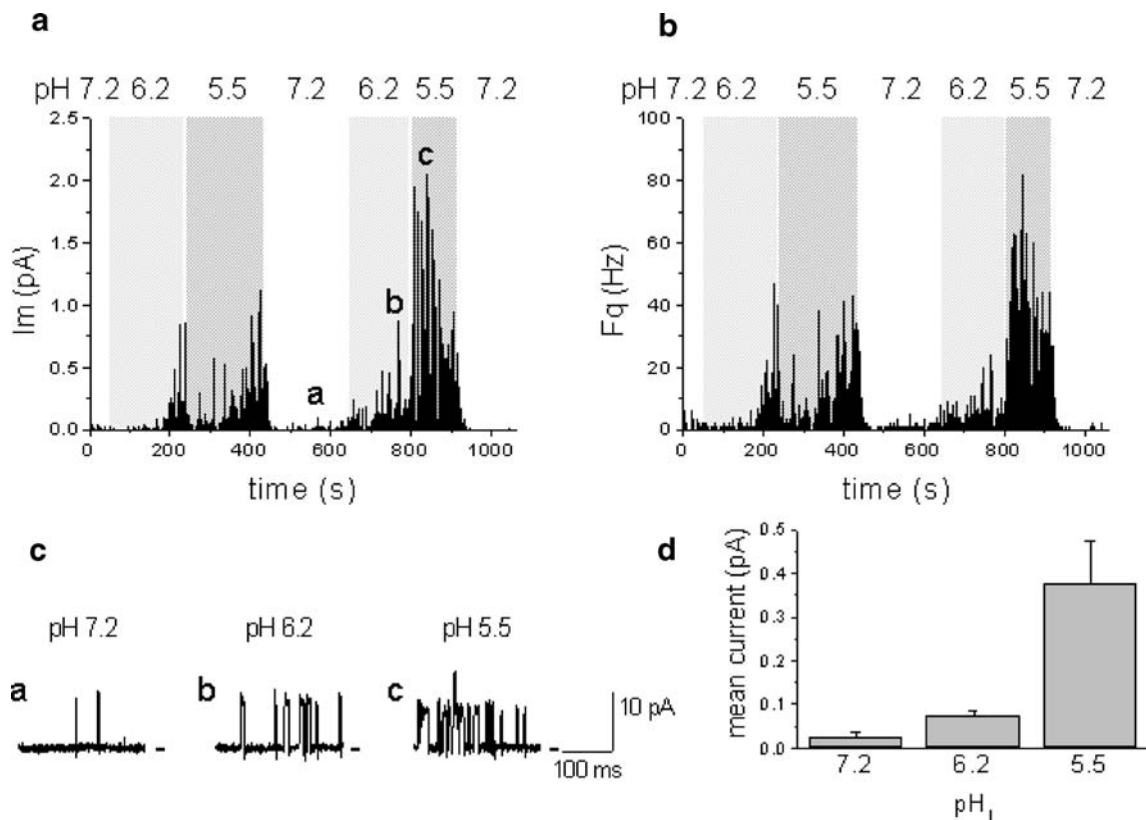
Fig. 2 Reversible effects of acidification on the activity of mechanosensitive channels in SL mode. The cytoplasmic face of a membrane patch containing two channels was perfused by Na^+ solutions at three different pH values and changed six times. *Columns in the plots* represent the mean patch current (**a**) and the opening frequency (**b**), calculated from 1-s-long consecutive data segments. The current traces in (**c**) are samples of activity at the times marked **a**, **b**, and **c**. The channel closed current is indicated by the *mark on the right* of each record. Outward currents are displayed as upward deflections. The membrane potential was held at +80 mV. Filtering 1 kHz. In (**d**), the mean values of the patch current measured on 20 patches are displayed. The means at the three pH values were significantly different ($p<0.05$, paired t test)
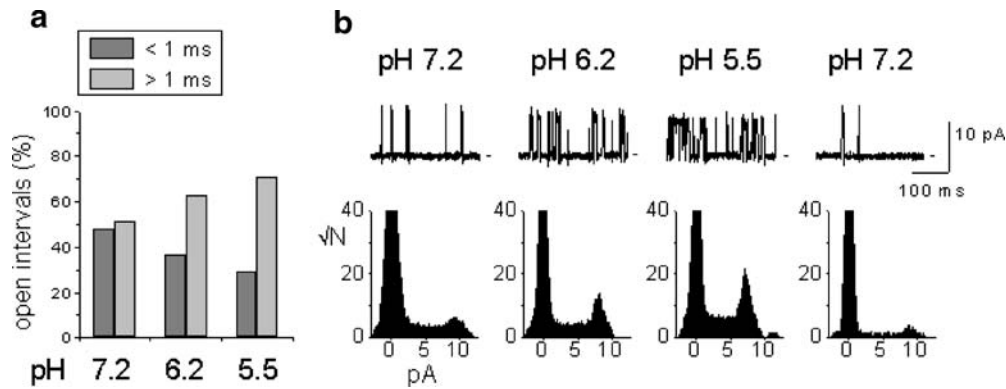
Fig. 3 Effects of acidification on the mean channel open time and the conductance of a single channel displaying the SL mode. **a** The open dwell times recorded in a representative experiment were grouped in two classes, containing dwell times shorter and longer than 1 ms, respectively. It can be noted that acidification increased the fraction of open intervals >1 ms. **b** Samples of activity recorded from a membrane patch containing a single channel at different values of pH. The membrane potential was held at +80 mV and the filtering at 1 kHz. The corresponding all points histograms are displayed at the *bottom*, and the single channel chord conductance values from left to right were 117.5, 102.5, 90.0 and 112.5 pS. A reversible decrease of single channel conductance was observed under low pH

demonstrated by patch clamp experiments combined with ion substitution [2]. However, to seek evidence that these channels can contribute to cytoplasmic calcium homeostasis in leech neurons, macroscopic changes in intracellular calcium concentration were imaged with fura-2, in an experimental condition where ions are in the physiological ratios. After loading of single neuron cell bodies with the calcium sensitive dye, ratiometric measurements were performed while cells were induced to swell by superfusion with hypotonic solution.

The upper graph of Fig. 7 illustrates the typical response to a 80-s hypotonic stimulation. After swelling, the cell shrinks again as shown by the time course of the trace with open circles, illustrating the value of the membrane area, as well as by the three images acquired at times a, b and c.

Intracellular calcium (trace with filled circles) starts rising when membrane area increases to about 10% of the initial value and then backtracks slowly. When a long stimulation was applied (8 min in the lower graph), the calcium signal reached a peak and then relaxed to a lower steady level.

The swelling-induced calcium response can be observed after a long superfusion with Ca^{2+}-free solution (Fig. 8a), and this response is presumably due to calcium release by internal stores. Preliminary experiments of light pressure stimulation with glass probes showed that these release responses, not involving calcium inflow through ion channels, exhibit the lowest threshold in imaging experiments (data not shown).

In standard leech saline, the temporary addition of Gd^{3+} and gentamicin after the peak of a swelling response
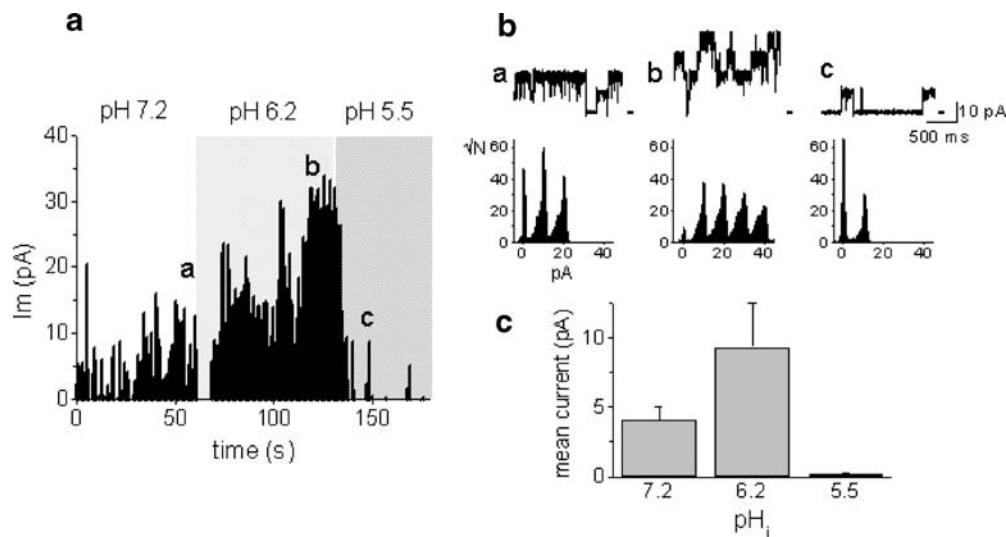
Fig. 4 Effect of acidification on the activity recorded from a patch containing four mechanosensitive channels in MC mode. **a** *Columns in the plot* represent the mean patch current calculated from 1-s-long consecutive data segments. **b** Samples of activity and corresponding all points histograms at the three values of pH, at the times marked **a**, **b**, and **c**. Holding membrane potential +80 mV, filtering 1 kHz. In (**c**), the mean values of the patch current measured on 11 patches exhibiting MC mode are displayed. The means at the three pH values were significantly different ($p<0.01$, paired t test)

Fig. 5 The reduction of calcium concentration induces a reversible decrease in the activity recorded from a patch containing two mechanosensitive channels in SL mode. **a** *Columns in the plot* represent the mean patch current calculated from 1-s-long consecutive data segments. **b** Samples of activity at the times marked **a**, **b**, and **c**. Holding membrane potential +80 mV, filtering 1 kHz. **c** Mean values (±SEM) of the patch current recorded from the same patch at different calcium concentrations
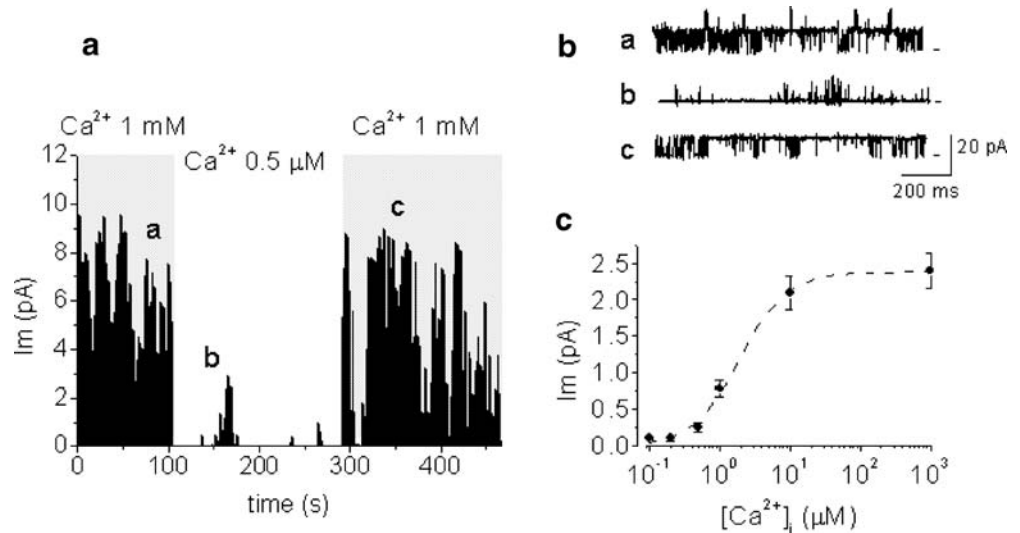
induced a reversible reduction of the calcium level (Fig. 8b). Three experiments performed with the same protocol of stimulation as that illustrated in Fig. 8b gave consistent results. Applying the opposite stimulation protocol that is a temporary removal of blockers (which were present at the beginning) after the peak response, a reversible rise in the calcium level was observed (Fig. 8c).

When gentamicin was used alone, it was able to mimic part of the effect observed with both blockers, but the increase of intracellular calcium was consistently transient (Fig. 8d). The results shown in Fig. 8d and c were seen in all eight experiments that used the same protocol, three with both blockers and five with gentamicin alone (Fig. 9). These experiments allowed us to visualize a gentamicin-sensitive contribution to the swelling-induced calcium

Fig. 6 Samples of current showing the effect of a reduction of calcium concentration in the solution bathing the internal face of a membrane patch containing two mechanosensitive channels. The lower calcium concentration abolishes the activity of the channel exhibiting the SL mode (*asterisk*), while it has no effect on the channel exhibiting the MC mode (*triangle*). The membrane potential was held at +80 mV, and the filtering was 1 kHz. Outward currents are displayed as upward deflection. The channel closed current is indicated by the *mark on the right* of each record
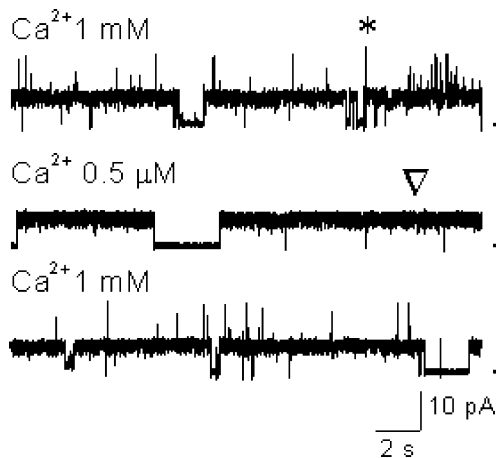
signal, presumably due to the activation of the mechanosensitive cation channels.

Discussion

Mechanosensitive ion channels detect and transduce external mechanical forces into electrical and chemical intracellular signals. They have been identified in a variety of cells using electrophysiological techniques; however, their molecular identification has been slowed, mainly by the lack of specific toxins. Because genetic screens in *Caenorhabditis* and *Drosophila* have identified genes involved in mechanosensation, the molecular characterization of mechanosensitive channels in eukaryotic cells has been greatly improved. Recent work has shown that mechanosensitive channels belong to unrelated molecular families, and that these channels are often affected by other stimuli, suggesting that a general strategy of cells in the course of evolution was to recruit for mechanotransduction members of existing families of channels [21, 34]. Well-characterized mechanosensitive channels in eukaryotes have been found among two pore-domain (2P) K^+ channels TREK and TRAAK [28], DEG/ENaC channels [36], and TRP cation channels [18].

One of the highlights of the function of the 2P-domain channels, in particular of TREK members, which have been extensively investigated, is their polymodal activation by a variety of intra- and extracellular stimuli, such as stretch, cell swelling, low intracellular pH, intracellular ATP, heat, polyunsaturated fatty acids, and volatile anesthetics [14, 19, 35]. This feature, along with their expression in the central nervous system, makes them remarkable molecular sensors suitable for integration of different stimuli to modulate the cell membrane potential.

An alternative strategy for providing cells with transducers of various internal and external environmental stimuli is the expression of multifunctional channels with a modular architecture. TRP channels fulfill such a function through alternative expression of a rich variety of cytoplasmic

Fig. 7 Typical calcium responses of a cultured cell body without arborization to short- (*upper graph*) and long-lasting (*lower graph*) hypotonic swelling (hpt). *Open circles* indicate the normalized membrane area values; *filled circles* represent the intracellular calcium concentrations. For details, see text
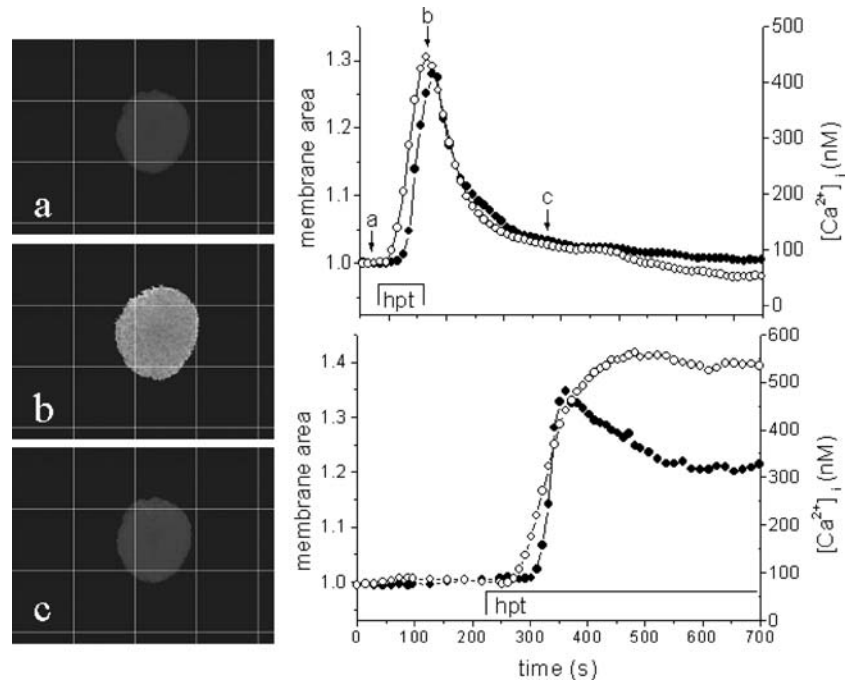
domains, as well as by heteromultimerization. In addition, these channels have been proposed to act as receptor and/or store-operated channels [32].

Although leech mechanosensitive channels were identified long ago [30], since then, no information concerning their molecular structure has been available. However, the biophysical and pharmacological properties of these channels appeared similar to those described in *Xenopus* oocytes, recently characterized as TRPC1 [20], and to the

typical mechanogated channels of vertebrate hair cells [33], structurally identified as TRPA1 [4]. Cation selectivity, calcium permeability, conductance of about 100 pS, blockage by Gd^{3+}, amiloride and gentamicin, mechanosensitivity and weak voltage-sensitivity [23, 33] are the shared features of leech and vertebrate hair cell mechanosensitive channels. Moreover, TRP channels have been found to be responsible for mechanotransduction in

Fig. 8 Representative calcium signals generated by hypotonic swelling of cultured cell bodies, in different experimental conditions. In Ca^{2+}-free solutions (**a**); with standard leech saline, in the presence (hpt+bl) and in the absence (hpt-bl) of mechanosensitive channel blockers (**b**, **c**); with (hpt+gnt) and without (hpt-gnt) gentamicin (**d**). For details, see text hpt: hypotonic, ist: isotonic, bl: blockers, gnt: gentamicin
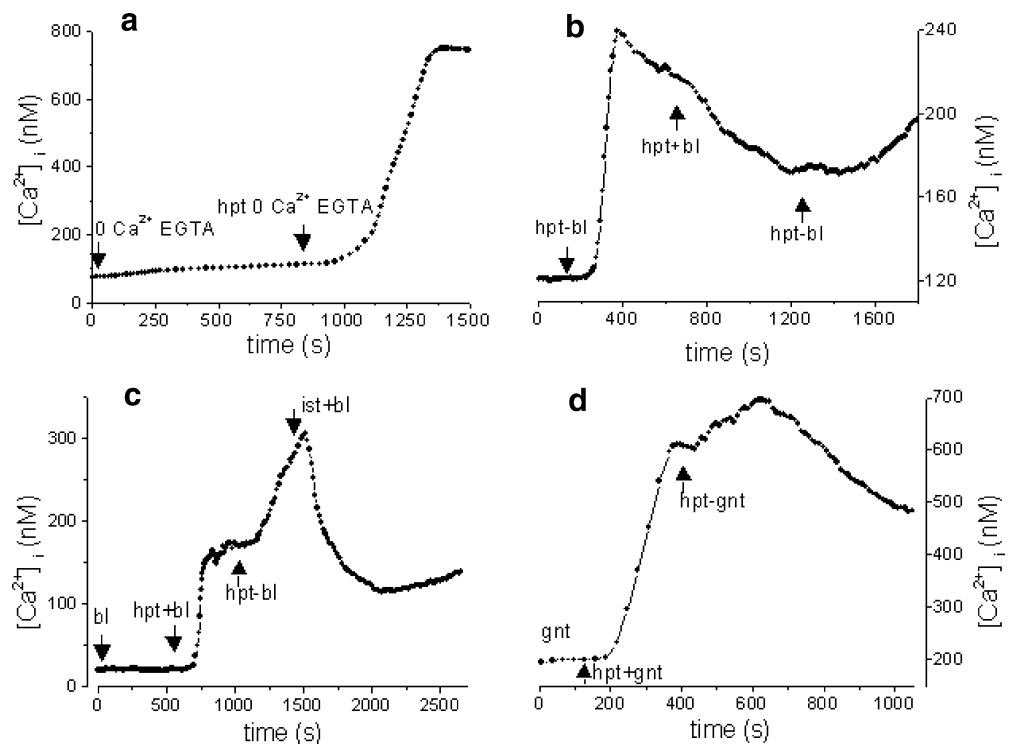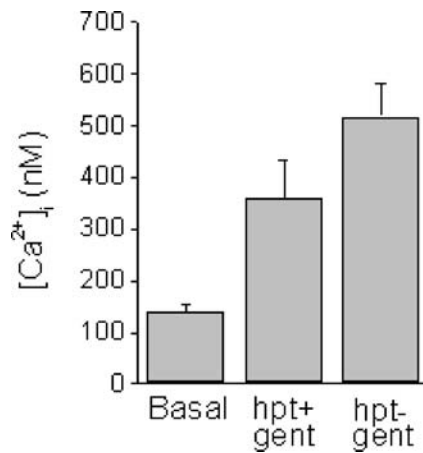
Fig. 9 Mean values (±SEM, *N*=5) of intracellular calcium in isolated neuronal cell bodies. The measurements were taken in the presence of 200 μM gentamicin (basal), after hypotonic swelling (hpt+gnt) and after removal of gentamicin (hpt-gnt). All means are significantly different (*p*<0.05). The experimental protocol is the same as that illustrated in Fig. 8d

invertebrates, as in bristle receptors of *Drosophila* and in polymodal sensory neurons of *Caenorhabditis* [26, 39].

Although, like TREKs, leech channels exhibit sensitivity to intracellular acidosis, SL and MC modes are not sensitive to polyunsaturated fatty acids (data not shown) and they are not K^+ selective. On the other hand, the high single-channel conductance in K^+ solutions [30] and the low sensitivity to amiloride [2] make it unlikely that leech mechanosensitive channels belong to the DEG/ENaC family [16, 25]. Rather, leech mechanosensitive channels have biophysical properties which are consistent with those of a TRP channel. However, the properties of the leech mechanosensitive cation channels do not entirely match those of known members of this family in other species. For example, some transient receptor potential (TRP) vanilloids that respond to cell swelling involve an indirect mode of activation [26, 38]; TRP melastatin channels, which are activated by intracellular calcium, are essentially impermeable to this ion and their mechanosensitivity is not reported [6, 37]. These findings stress the heterogeneity found in this channel family [3, 24].

As far as the different activation of leech channels in the two modes is concerned, we do not have a ready explanation for the different properties reported in this study. It should be noted that while the basic biophysical features of SL and MC unitary currents, such as main conductance, selectivity, and pharmacology, are indistinguishable, the two modes mainly differ in their sensitivity to environmental factors. Two hypotheses can be put forward: either the properties of the two modes might reflect a different subunit composition or they might be due to a different association with cytoskeletal, scaffolding, or modulation molecules.

Leech mechanosensitive channels are expressed in T and P sensory neurons which are involved in transduction of light touch or pressure stimuli applied to the skin [1], as well as in other neurons of the segmental ganglia such as motor neurons. Channel fast activation and deactivation to

pressure pulses are consistent with a direct gating mechanism and make these channels good candidates for the transduction channels of mechanosensory neurons. In all types of neurons, these channels may play a role in general functions, such as the control of cell volume regulation and of cell growth.

Previously, it was shown that leech mechanosensitive channels exhibit a high calcium conductance [2], and we have now found that they can contribute to the swelling-induced calcium signaling. The finding that the channels in SL mode can be activated by intracellular calcium with a threshold-saturation range of concentrations between 1 and 10 μM is of particular interest. These calcium concentration values are far from the resting level and can be probably reached when this ion is released by the internal stores. As expected, mechanosensitive leech channels are down-modulated at rest, to avoid cytotoxic effects, and they are recruited under calcium release. Although this does not appear as a true store-operated mechanism [27], it can attain the same goal. According to this scheme, leech mechanosensitive ion channels may work as amplifiers of neuronal calcium signals in two ways: (1) directly, through a calcium-induced activation of a calcium current, and (2) indirectly, by depolarization and consequent activation of voltage-gated calcium channels [8]. It has been reported that in different preparations, growth cones generate periodic elevations of intracellular calcium concentration as they migrate in vitro and in vivo, and the rate of axon growth is inversely proportional to the frequency of calcium transients [9]. Proposed models of calcium signaling in growth cone migration assign to TRPC channels the essential role of enhancing the calcium transients, as in the chemotropic turning behavior in response to netrin and BDNF [17, 31, 40].

It is interesting to note that the leech mechanosensitive channels are expressed in neuronal growth cones and previous findings showed that gentamicin increases axon outgrowth of leech neurons in culture [2], suggesting a role in cell growth. Leech mechanosensitive channels might be involved in cell growth by amplifying mechanically induced calcium signals.

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