INTRODUCTION

The mechanosensitivity of dorsal root ganglion (DRG) neurons in vivo has been demonstrated on different levels. Acute compression of DRG results in prolonged and slowly adapting repetitive firing (Howe et al. 1977) or an increased firing frequency (Wall and Devor 1983). Chronic compression is associated with elevated content of several neuropeptides in these neurons (Badalamente et al. 1987; Cornef jord et al. 1995; Kawakami et al. 1994), and numerous sensory disturbances are attributed to mechanical compression of the DRG in pathological conditions such as chronic disk herniation (Hanai et al. 1996; Howe et al. 1977; Kawakami et al. 1994). Yet the cellular basis of the mechanosensitivity in DRG neurons remains unknown.

While recording the effects of applied solutions on Ca$^{2+}$ currents in rat DRG neurons in culture, we observed consistent responses even when the solution flowing onto the cell from a small pipette was identical to the bath solution. Here we present evidence that the mechanical stimulation differentially affects two types of voltage-activated Ca$^{2+}$ channel, with corresponding changes in Ca$^{2+}$ influx during the action potential. Neither excitability nor membrane potential were altered directly by flow. Although not previously described in neurons, mechanosensitivity of voltage-activated Ca$^{2+}$ channels with some similar features has been reported in anterior pituitary somatotrophs (Ben-Tabou et al. 1994).
which comprises several current types. T-type current (peak amplitude 1.85 ± 0.52 nA, mean ± SD, n = 7) was isolated either by blocking the HVA current with 20 μM Cd²⁺ or by using cells where T-type current remained uncontaminated for several minutes after cell impalement because of a delayed run-up of HVA current. HVA current (peak amplitude 5.52 ± 4.55 (SD) nA, n = 13) was selected for study when contaminated with only a minor proportion of T-type, which was ignored in those cases. The N-type component of HVA Ca²⁺ current was blocked with 3 mM ω-conotoxin GVIA (ω-CgTx; Alomone Labs, Jerusalem, Israel).

Excitability was monitored by varying the amplitude of a 10-ms depolarizing current pulse in 2% steps to track the spike threshold (defined as the current amplitude required to evoke a spike with 50% success) with QTRAC software. Action-potential waveform was monitored by alternating the 10-ms tracking stimulus with a 50-ms current pulse of fixed, suprathreshold amplitude. Half-width was normally measured at 0.5 of spike amplitude, measured from the holding potential, with a range of 0.4–0.6 in a few cells to minimize noise. During these “current-clamp” recordings, the holding current was automatically adjusted between stimuli to maintain a membrane potential of −90 mV.

**Mechanical stimulation**

In pilot experiments, attempts were made to distort the cell membrane with the use of light suction through the recording micropipette, reduced osmolarity (by 10%) of the bath solution, or by a stream of bath solution from a pipette. Increases in HVA current were seen with each method, but the flow method (see Fig. 1A) was preferred as producing more reliably reversible effects. A flow of 0.2–1 ml min⁻¹ was applied to the cell through a polypropylene pipette tip (500-μm internal tip diameter) from a distance of ~500 μm. The response to flow was tested only on cells firmly attached to the dish. Although no obvious cell deformation or displacement could be detected at a magnification of ×400, enlarged images (×3,000–8,000) of nine cells before and during flow application revealed a small lateral movement of the soma in the direction of the flow, reaching a maximum of 2 μm. Shear force \( \tau \) (dyn cm⁻²) can be calculated according to the equation (Olesen et al. 1988)

\[
\tau = 4 \mu Q/r^2
\]

where \( \mu \) represents fluid viscosity (7.7 × 10⁻³ P), \( Q \), the flow rate (ml s⁻¹), and \( r \) the internal tip radius (cm). The typical shear force (calculated with \( Q = 0.5 \text{ ml min}^{-1} \)) was therefore 5.2 dyn cm⁻² at the tip and less at the level of the cell. The flow solution was always identical to the bath solution except during application of ω-CgTx.

**Data analysis**

Because calcium channel currents varied considerably in amplitude from cell to cell, the effects of flow on current amplitudes and on conductances were measured as percentage changes, and the significance of the differences in mean percentage change from zero was assessed by single sample, two-tailed t-tests. The effects of flow on the kinetics and voltage dependence of the Ba²⁺ currents were treated similarly (except that absolute rather than relative changes in half-activation voltage and equivalent gating charge were averaged).

**RESULTS**

DRG neurons that responded to flow of solution by a change in Ba²⁺ current or spike waveform had cell diameters ranging from 24 to 55 μm [32 ± 7.2 (SD) μm, n = 33]. The small fluctuations in the values of holding current, series resistance, and membrane capacitance throughout the recordings at a holding potential of −90 mV did not coincide with flow applications (mean values were 508 ± 80 pA, 5.9 ± 0.4 MΩ, and 35.5 ± 3.0 pF, respectively, SE, n = 20). In contrast, flow during repeated protocols of depolarizing voltage steps or ramps revealed modification of Ba²⁺ current amplitude, kinetics, or voltage dependence.

Voltage-clamp recordings were made in solutions with extracellular Ba²⁺ to increase current through Ca²⁺ channels and with extracellular TEA and intracellular Cs⁺ to reduce K⁺ currents. T-type currents were reduced by flow with a corresponding reduction but also distortion of the current-voltage relations (Fig. 1, B and C). The HVA current shown in Fig. 1D responded to flow by an amplitude increase as well as by a faster rise time. The current-voltage relation was also increased and distorted (Fig. 1E). To rule out nonmechanical effects of flow, such as DC shifts, on the recorded currents, a flow of solution away from the cell...
Flow effects on voltage dependence and kinetics of Ba\(^{2+}\) currents

The presence of several pharmacologically distinct HVA current types in DRG neurons is well established and includes at least the L, N, and P types, whereas the remaining unblocked portion suggests additional types (Mintz et al. 1992; Scroggs and Fox 1992a). The specific blocker of the N-type channel, \(\omega\)-CgTx, was found to block a large proportion of the HVA current (33 \(\pm\) 7\%, SE, \(n = 6\)), in agreement with previous studies of DRG neurons (Mintz et al. 1992; Piser et al. 1994; Scroggs and Fox 1992a). After the blockade of the \(\omega\)-CgTx-sensitive current, the amplitude of the remaining HVA current was no longer affected by flow (see Fig. 2B), indicating that the N-type current was involved in the increase of flow-induced Ba\(^{2+}\) influx.

To compare the effects of flow on different Ba\(^{2+}\) currents, we measured the percentage changes in several biophysical parameters. Although the mean increase in total HVA current in response to flow was significant and reached 23.3\% (range 3.0–98.7\%), the change after \(\omega\)-CgTx application was not significantly different from zero (range \(-5.2\%–4.7\%\), Fig. 3A). The T type was reduced significantly, on average by 13.5\% (Fig. 3A). These results could be largely accounted for by the similar changes in maximum conductance (Fig. 3B). The increase in N-type current was calculated to be 74 \(\pm\) 18\% (SE, \(n = 6\)), based on the proportion of the \(\omega\)-CgTx-sensitive current and the assumption that only this portion of the total HVA current responded to flow. No correlation was found between cell diameter and the relative magnitude of flow response in either HVA or T-type currents.}

**Flow effects on amplitude and conductance of Ba\(^{2+}\) currents**

HVA current appeared to respond to the flow stimulation by a small but significant hyperpolarizing shift of the activation curve [2.1 \(\pm\) 0.4 (SE) mV, \(n = 12\), \(P < 0.001\)] and

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**FIG. 2.** Augmentation of peak HVA Ba\(^{2+}\) current by flow and the blockade of the effect by \(\omega\)-CgTx. A: flow directed away from the cell had no effect (con flow). Direct flow (flow) increased peak current by 19.5\% and was followed by amplitude restoration whereas a 2nd flow application elicited a typically smaller response. B: flow in another neuron elicited a 15.5\% increase in peak current. After blockade of N-type current with 3 \(\mu\)M \(\omega\)-CgTx (which blocked >50\% of the total current) an identical flow application failed to increase the remaining current. Currents in A & B were elicited with voltage ramps at 30-s interval and in B with voltage steps at 15-s interval (for details, see Fig. 1). Horizontal bars indicate timing of 0.05, respectively — flow or drug application.

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**FIG. 3.** Changes in amplitude and conductance of Ba\(^{2+}\) currents in response to flow. Bars in each panel represent the average change of HVA current in response to direct flow (\(n = 12–13\)) and unblocked portion suggests additional types (Mintz et al. 1992; Scroggs and Fox 1992a). The specific blocker of the N-type channel, \(\omega\)-CgTx, was found to block a large proportion of the HVA current (33 \(\pm\) 7\%, SE, \(n = 6\)), in agreement with previous studies of DRG neurons (Mintz et al. 1992; Piser et al. 1994; Scroggs and Fox 1992a). After the blockade of the \(\omega\)-CgTx-sensitive current, the amplitude of the remaining HVA current was no longer affected by flow (see Fig. 2B), indicating that the N-type current was involved in the increase of flow-induced Ba\(^{2+}\) influx.

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**Flow effects on voltage dependence and kinetics of Ba\(^{2+}\) currents**

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by an increase in the mean equivalent gating charge (calculated from the slope factor) of 0.8 ± 0.2 (n = 12, P < 0.002). Flow also appeared to affect the kinetics of Ba2+ currents. The rise time (defined as the time between 10 and 90% of the current rising phase) of the total HVA current was decreased by 23.9 ± 3.5% (n = 13, P < 0.0001), whereas the T-type kinetics were less sensitive to flow, with a rise time dropping by 7.6 ± 2.9% (n = 7, P < 0.05). When control (indirect) flow was applied none of the changes in HVA current characteristics was significant. While in ω-CgTx, flow still induced a significant drop in HVA current (14.5 ± 4.6%, n = 6, P < 0.05) but eliminated all the other flow-induced changes of the HVA current. To test whether these changes in voltage dependence and kinetics could be due to the jet-induced changes in current amplitude and therefore in series-resistance error, the HVA activation curves of 10 cells were recalculated with a corrected voltage error (based on the product of the current and the residual uncompensated fraction of the series resistance read from the front panel potentiometer for each cell). The results showed that although the increase in maximum conductance was not appreciably affected by the voltage correction, an average of 36% of the shift in activation curve and 41% of the change in equivalent gating charge could be accounted for by the increased series resistance error.

Flow effects on excitability and action potentials

Current-clamp recordings were made on 51 neurons in the modified physiological solution, buffered with HEPES to avoid possible differences in pH between bath and flow solution that might arise with a CO2/HCO3 buffer. All neurons generated all-or-none action potentials when their resting membrane potentials were held at −90 mV by applied current. This enabled excitability to be monitored automatically by tracking the threshold amplitude of a 10-ms current pulse required to evoke a spike. Action potentials elicited by 50-ms suprathreshold current pulses varied considerably in shape, with half-widths varying from 1 to 12 ms. Forty-four neurons (86%) had a clear shoulder on the repolarizing phase of their action potentials, indicating an activation of Ca2+ currents (e.g., Fig. 4, A and E), whereas the remainder had narrow spikes with no shoulder. When mechanically stimulated by flow of the bath solution, no effects were seen on electrical excitability, on the holding current, or on spike amplitude. However, 12% of neurons exhibited a reversible increase in spike duration (Fig. 4, A–D), 27% exhibited a reversible decrease (Fig. 4, E and F), and the remaining 61% showed no change or a change of <5%. Only action potentials with a clear shoulder were affected by flow, and the two flow-responding groups differed in control spike duration (P < 0.05, 2-tailed t-test, df = 19). In the first group spike duration increased from an average of 8.1 to 10.6 ms (change = 33 ± 8%, SE, n = 6) with rapid onset and slow recovery (time constant 30–90 s). In the second group spike duration decreased from an average of 5.2 to 4.8 ms (change = −8.4 ± 1.1%, n = 14) with rapid onset and recovery. The nonresponding group included neurons with action potentials of similar shape and duration to those in the flow-responding groups as well as 23% with shorter action potentials without a shoulder. Cell diameter did not differ significantly between the two flow-responding groups,
and within the two groups there was no correlation between cell diameter and the relative magnitude of either spike broadening or spike narrowing ($r = -0.15$, $df = 4$, $P > 0.05$; $r = -0.06$, $df = 12$, $P > 0.05$, respectively). Flow-induced increases in spike duration were blocked after application of $\omega$-CgTx (not shown), whereas the decreases in spike duration persisted in Cd$^{2+}$ (Fig. 4F) and in $\omega$-CgTx. These results are consistent with the voltage-clamp results and indicate that the flow-induced spike duration increases were mediated via $\omega$-CgTx-sensitive channels, whereas the spike duration decreases were most likely mediated by T-type channels. In two neurons action-potential prolongation was also accompanied by a larger amplitude of afterhyperpolarization.

**Discussion**

We report that two types of voltage-activated Ca$^{2+}$ currents were modified by a flow of bath solution. The lack of effect of the control flow, combined with the specific and opposite effects of direct flow on the T- versus the N-type current, rules out the possibility of some nonmechanical effects of flow, such as DC shifts, changes in ionic concentration at the cell surface, or contamination. The possibility that cell movement altered the area of membrane and numbers of channels exposed was ruled out both by the absence of changes in excitability or leak currents and by the opposite effects on different Ca$^{2+}$ currents. It is conceivable that the flow removes from the cell surface an autoreleased factor that modulates Ca$^{2+}$ channels, but we are unaware of any reports of such an action, and our preliminary evidence that negative pressure and hypoosmotic solutions can produce similar effects suggests that membrane distortion is involved. We therefore consider it most likely that the changes in amplitude of voltage-activated Ca$^{2+}$ currents are due to the shear stress arising from the flow of solution against the cell surface, possibly mediated via the cytoskeleton (Ingber 1997). Previous studies suggested interactions between the cytoskeleton and the function of voltage-activated Ca$^{2+}$ channels; in snail ganglia the cytoskeleton was implicated in both metabolic dependence and Ca$^{2+}$-dependent inactivation of Ca$^{2+}$ channels (Johnson and Byrely 1993), and in guinea-pig gastric myocytes the role of actin microfilaments was demonstrated in osmotic stretch-induced modulation of Ca$^{2+}$ currents (Xu et al. 1997). K$^+$ channels, such as inward-rectifying (Burton and Hutter 1990) and Ca$^{2+}$-activated channels (reviewed in Sackin 1995), were also reported to respond to mechanical stimulation of the membrane in nonneuronal preparations.

**Mechanosensitivity of T- and N-type Ca$^{2+}$ channels**

The flow-induced augmentation of total HVA current was abolished in the presence of $\omega$-CgTx and therefore indicates that N-type current constitutes the major component that mediates the observed mechanosensitivity. Both the flow-induced increase in N-type current and the decrease in T-type current (evoked by a voltage step from $-90$ to $-20$ mV) could mostly be accounted for by the corresponding changes in maximum conductance. An additional contribution to the modulation of HVA current amplitude may involve the observed changes in voltage dependence, i.e., the hyperpolarizing shift and the steepness increase in activation curve, especially in the dynamic range where conductance increases e-fold per $<4$ mV. The flow of solution resulted also in a faster rise time of HVA current. However, the apparent shifts in voltage dependence and kinetics of the N-type current could partly be accounted for by the increased series resistance error, as the recalculations showed. Nevertheless, a mechanical effect on these parameters could not be ruled out completely, and, in the case of the T-type current, where flow application also resulted in faster rise time despite a reduced voltage error (because of the flow-induced current decrease), a drop in series-resistance error could not have contributed to this change.

**Comparison with other flow-dependent currents**

Modulation of channel gating by flow was shown before in inward-rectifying K$^+$ channels of skeletal muscles (Burton and Hutter 1990). In that case, the channels were affected by flow past the inner surface of the membrane in inside-out patches (but not by stretch), and activation and deactivation appeared instantaneous. The case therefore appears very different, but a common factor could be movement of cytoskeletal elements attached to the channels. A closer parallel is the suppression of LVA and HVA Ca$^{2+}$ currents by flow in rat anterior pituitary somatotrophs, reported by Ben-Tabou et al. (1994). They estimated a similar shear stress (3.77 dyn cm$^{-2}$) but for a slower flow rate from a much smaller pipette. Although the effects they observed on HVA currents were the opposite to those in DRG neurons and the reductions in LVA current were much greater (averaging 58%), there was a strikingly similar time course (see their Fig. 1), suggesting a common mechanism; the onset of flow changed the Ca$^{2+}$ current within seconds, whereas recovery took a few minutes.

**Mechanosensitivity of DRG neurons in physiological conditions**

In view of the reported mechanosensitivity of DRG neurons in vivo (Howe et al. 1977; Wall and Devor 1983) and the involvement of specialized stretch-activated channels in the mechanoelectrical transduction in a variety of sensory neurons (Morris 1990; Sackin 1995), the absence of any detectable effect of flow on electrical excitability or holding current was striking. Neither Na$^+$ channels nor leakage or K$^+$ channels active at the resting potential were affected by this type of mechanical stimulation. Flow-induced changes in the waveform of evoked action potentials were restricted to Ca$^{2+}$-current-containing spikes and were blocked selectively in agreement with the Ba$^{2+}$ current results. Spike duration increases were not nearly as common as the augmentations of HVA currents seen in the voltage-clamp experiments. The different solutions used may explain part of this discrepancy, but it is most likely that different proportions of the various Ca$^{2+}$ current types were activated by action potentials in different neurons. Although the voltage-step protocol was uniform, action-potential waveforms in DRG neurons vary considerably (Scroggs and Fox 1992b). The possible presence of T- as well as N-type currents, with their opposite responses to flow, in the same cell may also contribute to the relatively low occurrence of spike duration.
changes. The strong intracellular Ca\(^{2+}\) buffering used in these experiments would limit the effects of altered Ca\(^{2+}\) influx on Ca\(^{2+}\)-dependent K\(^+\) current, and hence on excitability.

**Possible consequences of Ca\(^{2+}\) channel mechanosensitivity in sensory neurons**

A physiological function of voltage-activated Ca\(^{2+}\) channel mechanosensitivity in DRG neurons is unclear because excitability was not directly affected, and these cells are normally not subjected to mechanical stress. Our evidence that Ca\(^{2+}\) influx during action potentials is mechanosensitive in a proportion of cells provides a means by which this property can modulate intracellular Ca\(^{2+}\) and hence other functions. For example, Huang and Neher (1996) recently demonstrated that exocytosis of substance P from DRG cell somata is exquisitely sensitive to the level of intracellular free Ca\(^{2+}\) and suggested that it may play a critical role in modulating neuronal excitability. The modulation of calcium current that we demonstrated is modest, compared with what could be achieved by modulating the discharge rate of the cells. However, the degree of cell deformation that appeared responsible for the effects we recorded was also exceedingly modest compared with the gross flattening of DRGs and distortion of neurons seen in autopsy material from herniated disk cases (Lindblom and Rexed 1948).

In conclusion, mechanical stimulation by flow of bath solution was found to modulate two voltage-activated Ca\(^{2+}\) channels in DRG neurons. This constitutes a previously undescribed route by which mechanical events can affect Ca\(^{2+}\) influx in sensory neurons. If mechanical enhancement of Ca\(^{2+}\) entry also occurs chronically in vivo, it could contribute to the sensory disturbances of disk herniation.

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