Capsaicin alters human $Na_V1.5$ mechanosensitivity

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Running Head: Alteration of $Na_V1.5$ mechanosensitivity by capsaicin

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ABSTRACT

SCN5A-encoded Nav1.5 is a voltage-gated Na^+ channel expressed in cardiac myocytes and human gastrointestinal (GI) smooth muscle cells (SMCs). Nav1.5 contributes to electrical excitability in the heart and slow waves in the gut. Nav1.5 is also mechanosensitive, and mechanical force modulates several modes of Nav1.5's voltage-dependent function. Nav1.5 mutations in patients with cardiac arrhythmias and gastrointestinal diseases lead to abnormal mechano- and voltage-sensitivity. Membrane permeable amphipathic drugs that target Nav1.5 in the heart and GI tract alter Nav1.5 mechanosensitivity (MS), suggesting that amphipaths may be a viable therapeutic option for modulating Nav1.5 function. We therefore searched for membrane-permeable amphipathic agents that would modulate Nav1.5 MS with minimal effect on Nav1.5 voltage-gating intact to more selectively target mechanosensitivity. We used two methods to assess Nav1.5 MS: (1) membrane suction in cell-attached macroscopic patches and (2) fluid shear stress on whole cells. We tested the effect of capsaicin on Nav1.5 MS by examining macropatch and whole-cell Na^+ current parameters with and without force. The pressure- and shear-mediated peak current increase and acceleration were effectively abolished by capsaicin. Capsaicin abolished the mechanosensitive shifts in the voltage-dependence of activation (shear) and inactivation (pressure and shear). Exploring the recovery from inactivation and use-dependent entry into inactivation, we found divergent stimulus-dependent effects that could potentiate or mitigate the effect of capsaicin, suggesting that mechanical stimuli may differentially modulate Nav1.5 MS. We conclude that selective modulation of MS makes capsaicin is a novel modulator of Nav1.5 MS and a promising therapeutic candidate.

65 Keywords: amphipathic, arrhythmia, capsaicin, electrophysiology, functional gastrointestinal
66 disorder, ion channel, irritable bowel syndrome, mechanosensitivity, mechanotransduction,
67 voltage-gated sodium channel type 5

68

INTRODUCTION

The S*CN5A*-encoded voltage-gated sodium channel Nav1.5 is mechanosensitive; mechanical force modulates Nav1.5's voltage-dependent function. This property is particularly relevant given that Nav1.5 is expressed in mechanically active tissues like the heart and gastrointestinal tract, where it contributes to electrical excitability in cardiac myocytes (CMs) and slow waves in smooth muscle cells (SMCs), respectively.[1] At the cellular level, mechanosensitive channels like Nav1.5 detect mechanical stimuli through lipid bilayer tension and/or cytoskeletal deformation.[2,3] In patch-clamp studies, mechanical stimuli in the form of membrane stretch and fluid shear stress modulate Nav1.5's voltage-dependent function by increasing whole-cell conductance, shifting voltage-dependence to hyperpolarized potentials, and accelerating its activation and inactivation kinetics. Therefore, in the context of cardiac and intestinal smooth muscle tissues, Nav1.5 mechanosensitivity (MS) has important implications.[1]

Channel-related disorders in common cardiac and gastrointestinal diseases, channelopathies[4, 5] are strongly linked to *SCN5A* mutations.[6, 7] Many *SCN5A* mutations lead to voltage-gating abnormalities, some associated with abnormal responses to mechanical stimuli.[7-10] Impaired stretch modulation in Nav1.5, for example, occurs in some mutations that also cause LQT3-type cardiac arrhythmias,[8, 11] and some mutations that lead to altered Nav1.5 MS are found in patients with IBS.[10, 12]

Because of their involvement in many diseases, ion channels are prime targets for pharmacological treatment.[13] Drugs for cardiac diseases may modulate the behavior of sodium channels like Nav1.5 and influence mechanosensitivity.[13-15] Yet, how membrane-permeable amphipathic drugs alter the mechanosensitivity of voltage-gated channels like Nav1.5, or whether they do so by a mechanism separate from Na^+ current inhibition, remain critical unanswered

questions.[13-15] For example, the membrane-permeable, amphipathic local anesthetic lidocaine inhibits peak current while inhibiting Nav1.5 mechanosensitivity at lower concentrations.[13, 14] Suggesting separate mechanisms for current inhibition and altered mechanosensation by amphipaths, the anesthetic binding site mutation F1760A[13] eliminates the voltage-dependent inhibition by lidocaine without altering lidocaine's effect on mechanosensitivity. The membrane-impermeant lidocaine analog, QX-314, in contrast had no effect on mechanosensitivity.

We therefore searched for membrane-permeable amphipathic agents with minimal effects on Nav1.5 voltage-gating for their impact on Nav1.5 mechanosensitivity. Among the candidates, capsaicin shows promise, and we characterized its ability to selectively modulate $Na_V1.5$ mechanosensitivity.

METHODS

Heterologous expression and cell culture

Wild-type *SCN5A* (Q1077del Nav1.5)[16] was co-transfected with pEGFP-C1 into HEK-293 cells with Lipofectamine 3000 reagent (Thermo Fisher Scientific, Massachusetts, USA).

Electrophysiology

Pipette fabrication. For whole-cell experiments, electrodes were pulled on a P-97 puller (Sutter Instruments, CA) from KG12 glass to a resistance of 2-5 MΩ. For cell-attached patch experiments, electrodes were pulled from 8250 glass (King Precision Glass, California, USA) then fire-polished to wide-bore, bullet-shaped tips with a final resistance of 1-2 MΩ. Electrodes were coated with R6101 elastomer (Dow Corning, MI) then cured by a heat gun to reduce capacitive transients.

Data acquisition. Whole-cell and cell-attached patch data from HEK-293 cells were recorded at 20 kHz with an Axopatch 200B patch-clamp amplifier, Digidata 1550, and pClamp11 software (Molecular Devices, CA).

Cell-attached patch. *Solutions.* The pipette solution contained (in mM): 150 Na^+, 160 Cl^-, 5 K^+, 2.5 $Ca2^+$, 10 HEPES, and 5.5 glucose with an osmolality of 305 mmol/kg and pH of 7.35. $GdCl_3$ (10 μM) was included in the pipette solution to inhibit endogenous stretch-activated channels. The bath solution contained (in mM): 15 Na^+, 140 Cs^+, 160 Cl^-, 2.5 Ca^{2+}, 5 K^+, 10 HEPES, and 5.5 glucose with an osmolality of 305 mmol/kg and pH of 7.35. Where applicable, capsaicin was diluted 1000-fold in bath solution from a 20 mM ethanol stock then added to the recording chamber. Seal pressures were digitally controlled and monitored by High-Speed Pressure Clamp (HSPC-2, ALA Scientific, NY). Suction ≤10 mmHg was applied to establish giga-seals. *Episodic protocol and mechanical stimulation by pressure.* Na^+ currents in macroscopic

patches were elicited by an identical pair of voltage ladders with 31-ms pressure steps up to -50 mmHg encompassing the second voltage ladder. Patches were held at +100 mV, stepped briefly for 10 ms to +190 mV to close Nav channels, then stepped through a 10-step voltage ladder from +100 to 0 mV in 21-ms long, 10-mV increments with a total sweep duration (equivalent to the interpulse interval) of 280 ms. Recordings were an average of 5 runs. Capsaicin (20 μM) was added to the chamber 5 min before testing the effects of the drug. *Recovery from inactivation:* To test the effect of pressure on the recovery of Nav1.5 from inactivation, cells were held at 120 mV and stepped to (1) 20 mV for 30 ms, next to (2) 120 mV for a variable duration to recover, then to (2) 20 mV for 30 ms. The time between the beginning of each sweep was 5 s. The length of the recovery time in stage (2) was varied between 1 and 300 ms, with half-log unit increments. The pressure step per sweep was 400 ms regardless of recovery time. *Use-dependent inactivation:* To test the effect of pressure on the onset (use dependence) of Nav1.5 inactivation, cells were held at 120 mV and stepped 20 times to 20 mV, and the frequency between steps was 33.33 to 3.33 Hz. The pressure step per sweep was 30 ms.

Whole-cell voltage clamp. *Solutions.* The intracellular solution contained (in mM): 145 Cs^+, 125 $CH_3SO_3^-$, 35 Cl^-, 5 Na^+, 5 Mg^{2+}, 10 HEPES, and 2 EGTA with an osmolality of 290 mmol/kg and pH of 7.0. The extracellular solution contained (in mM): 15 Na^+, 140 Cs^+, 160 Cl^-, 2.5 Ca^{2+}, 5 K^+, 10 HEPES, and 5.5 glucose with an osmolality of 290 mmol/kg and pH of 7.35. *Peak current, voltage dependence of activation, and kinetics of activation and inactivation:* To measure peak Na^+ current density, cells transfected with Nav1.5 were held at -120 mV then pulsed through a 2-stage, 19-step voltage ladder (1) from -110 to -30 mV in 5 mV intervals for 2.9 s each and (2) to -30 mV for 100 ms. The time from the start of each sweep to the next was 5 s. Peak currents at each voltage step were normalized to either the cell capacitance (pF) or the maximum

peak inward current without shear. *Recovery from inactivation:* Recovery from inactivation was measured by holding cells at -130 mV and the pulsed through a 3-stage, 10-step protocol to (1) -30 mV for 100 ms, next to (2) -130 mV for a variable duration to recover, then to (2) -30 mV for 100 ms. The time between sweep starts was 2.5 s. The length of the recovery time in stage (2) of sweep n was $4*2^n$ ms for a total of n = 10 sweeps. *Use-dependent inactivation:* To measure the onset of Nav1.5 inactivation, cells were held at -130 mV and stepped 10 times to -40 mV, in which the frequency of steps recorded ranged between 0.3 and 50 Hz. *Mechanical stimulation by shear stress.* When testing the effect of shear stress, the extracellular (bath) solution was perfused by gravity drip (at 10 mL/min) for the duration of the voltage protocol.

Data Analysis

The maximum peak Na^+ current and voltage dependence of activation were determined by fitting the Nav1.5 current-voltage (I-V) plots with $I_V=G_{MAX}*(V-E_{REV})/(1+e^{(V-V1/2A)/slope})$, where G_{MAX} is the maximum Na^+ conductance in whole cells (I_{MAX} is the maximum Na^+ current in patches), and $V_{1/2A}$ is the voltage of half-activation. Activation kinetics were determined by fitting currents with a two-term weighted exponential function: $I(t)=A_1e^{(-t/\tau A)}+ A_2e^{(-t/\tau I)}$, where τ_A and τ_I are the time constants of activation and inactivation, respectively, and A_X and C were constants. Steady-state inactivation was obtained by fitting available peak Na^+ currents with a 3-parameter sigmoid curve: $I_V=1/(1+e^{((V-V1/2I)/\delta VI)})$, where $V_{1/2I}$ is the half-point of steady-state inactivation (availability), and δV_I the slope. To determine the recovery from inactivation, peak Na^+ currents were fit with the equation: $I/I_0=1/(1+t/t_{1/2})^b$, where I/I_0 is the ratio of Na^+ current recovered following inactivation from the control current, b the rate of inactivation recovery, and $t_{1/2}$ the time where half of the Na^+ current has recovered from inactivation. Use-dependent inactivation was estimated by fitting the peak Na^+ currents of successive pulses with the equation: $I_{10}/I_1=I_fe^{b/(x+c)}$,

where I_{10}/I_1 is the peak Na^+ current in step 10 normalized to the peak of step 1, and I_f the maximally inactivated peak Na^+ current at frequency f, and b or c is the rate or constant of use-dependent inhibition, respectively. To calculate the half-frequency of use-dependent inactivation, I_f was plotted *vs.* pulse frequency f and fit with $I_f=(1-a)/(1+e^{(f1/2-f)/b})$, where a is limit of use-dependent inhibition, $f_{1/2}$ the half frequency of use-dependent inhibition, and b the slope. Data are expressed as the mean ± standard error of the mean (SEM). Significance was tested using a 2-way ANOVA with Tukey post-test, in which $P<0.05$ when comparing force to rest or capsaicin to drug-free.

RESULTS

Screen of amphipathic membrane-permeable drugs

We examined select amphipathic agents with high partition coefficients (Table 1) as potential modulators of Nav1.5 voltage dependence.[17-20] We tested each compound (10^{-9} to 10^{-4} M) for its ability to inhibit peak voltage-gated Na^+ currents (Figure 1A-D). Of the agents tested, Triton X-100 (logP_{OW} 4.6, IC_{50} 5.3 μM, Figure 1C-D), was the most potent and capsaicin (logP_{OW} 3.04, IC_{50} 60.2 μM, Figure 1C-D, Table 1) the least. The antiarrythmic amiodarone (logP_{OW} 7.2, IC_{50} 8.4 μM, Figure 1C-D) and β-blocker propranolol (logP_{OW} 3.48, IC_{50} 7.6 μM, Figure 1C-D) also inhibited Nav1.5 voltage-gating. Propranolol, which had a partition coefficient (logP_{OW}) similar to capsaicin, was an 8-fold more potent $Na_V1.5$ inhibitor than the latter, indicating that logP_{OW} is not a good predictor of the drug's effect on Nav1.5 voltage-gated function. Because our goal was to test mechanosensitivity while minimizing Nav1.5 voltage-dependent gating inhibition, we chose capsaicin (20 μM) for further investigation, as this dose inhibited voltage-dependent Na^+ current by ≤25% without mechano stimulus (Figure 1B-D).

Capsaicin inhibits increases in peak current and acceleration with mechanical stimuli

To test the effect of capsaicin on Nav1.5 mechanosensitivity, we used two complementary approaches for mechanical stimulation: (1) cell-attached macroscopic patches with suction and (2) whole-cell configuration with fluid shear stress (Tables 2-3, Figure 2). These complementary techniques allow us to highlight different aspects of the channels' mechanosensitivity due to both techniques' intrinsic strengths and weaknesses.[11, 21-23] The effect of pressure was tested in a pair-wise fashion[1, 11, 14], with pressure at 0 or -30 mmHg applied at each voltage step (Figure 2A,C,E-H). Whole-cell current responses to shear was tested by perfusion at 0 or 10 mL/min (Figure 2B,D,E-H). We then reassessed function in both configurations in the presence of 20 μM capsaicin

(Tables 2-3, Figure 2A-H). Suction increased normalized peak currents (I_{MAX}) by 16.6±2.4% (P<0.05; n = 24; Figure 2A,C,E), and shear increased the peak current (I_{PEAK}) by 16.0±3.1% in whole cells (0.26±0.10 nS increase in conductance; P<0.05, 0 to 10 mL/min; n = 12; Figure 2B,C,E). Capsaicin decreased I_{PEAK} by 22.1±3.9% (P<0.05, 0 to 20 μM capsaicin), and both pressure (+4.8±3.0%) and shear sensitivity (+3.1±3.8%, +0.08±0.05 nS) were lost (n = 12-14, P>0.05 to drug with no force).

In the absence of drug, pressure and shear accelerated Na^+ current activation, decreasing the activation constant (τ_{ACT}) by 20.0±5.3% or 20.4±3.3%, respectively (n = 12-14, P<0.05 to no force controls; Figure 2F). Capsaicin accelerated $Na_V1.5$ activation by 20.3±6.9% at rest (n = 12-14, P<0.05, 0 to 20 μM capsaicin) in whole cells but not in patches, and capsaicin inhibited the acceleration of activation induced by pressure and shear, as τ_{ACT} did not accelerate with pressure or shear (-11.0±5.4% or -1.3±7.0%, respectively; n = 12-14, P>0.05 to drug with no force, Figure 2F). Our results thus show that capsaicin inhibits the mechanosensitivity of peak current and kinetics of $Na_V1.5$ in both experimental configurations.

Capsaicin inhibits mechanically induced hyperpolarizing shifts in the voltage dependence of activation and availability

Pressure[1, 13, 14, 24] and shear[3, 11, 12] produce hyperpolarizing shifts in the voltage dependence of $Na_V1.5$ activation and inactivation. Membrane-permeable amphipathic drugs like lidocaine and ranolazine reduce these mechanosensitive shifts in voltage dependence.[13, 14] Therefore, we explored whether capsaicin could reduce the pressure- or shear-induced shifts in voltage dependence. Like in our previous work without drug[13, 14], suction (-30 mmHg) produced a leftward shift of -4.5±0.6 mV, and shear stress induced a smaller but significant shift of -1.5±0.6 mV in the voltage dependence of activation ($V_{1/2A}$) (P<0.05 to no force) (Table 2, Table 3, Figure 2C, G).

Without force, capsaicin produced a hyperpolarized shift in $V_{1/2A}$ (-1.6±0.4 mV; P<0.05, 0 to 20 μM capsaicin) in whole cells. With force, capsaicin inhibited the shear-induced shift in $V_{1/2A}$ (-0.3±0.1 mV, P>0.05 to drug with no shear) but not the pressure-induced shift (-2.4±0.6 mV, P<0.05 to drug with no pressure). Similar to shear-induced shifts in $V_{1/2A}$, pressure or shear shifted the voltage dependence of inactivation or availability ($V_{1/2I}$) in the absence of capsaicin (-6.0±0.9 mV with pressure or -2.5±0.9 mV with shear, P<0.05 to no force) (Tables 2-3, Figure 2D,H). Without force, capsaicin produced a hyperpolarizing shift in whole-cell $V_{1/2I}$ by -5.1±0.7 mV, as previously observed[25]. In the presence of capsaicin, however, neither pressure nor shear had a significant effect on $V_{1/2I}$ (-1.4±1.2 or -0.5±0.6 mV change, respectively, P>0.05 to drug with no force), suggesting loss of the MS of $Na_V1.5$ inactivation. Overall, our results show that capsaicin inhibited the mechanosensitive shifts in $Na_V1.5$ gating.

Effects of capsaicin and mechanical stimuli on recovery from inactivation

Both capsaicin and pressure delay recovery of $Na_V1.5$ from fast inactivation.[1, 25] Therefore, we tested whether the presence of capsaicin affected the recovery from fast inactivation (1 to 1000 ms) in the absence or presence of mechanical stimuli (Tables 2-3, Figure 3A-B). Without force or drug, Na^+ currents recovered within ~100 ms in either configuration (Figure 3C); the half-time of $Na_V1.5$ inactivation recovery ($t_{1/2R}$) at rest was 13.2±2.5 ms in the patch and 18.8±1.7 ms in whole-cell (Tables 2-3, Figure 3C-F). In addition, unlike the consistent responses to force regardless of stimulus or configuration described above, here we observed consistent differences between the two approaches. Shear accelerated $Na_V1.5$ $t_{1/2R}$ by 2.2±0.6 ms (P<0.05, 0 to 10 mL/min), whereas pressure delayed the $t_{1/2R}$ (+8.9±3.9 ms, P<0.05, 0 to -30 mmHg) (Tables 2-3, Figure 3C-F). In whole cells, without force, capsaicin delayed the recovery from inactivation; $t_{1/2R}$ increased from 18.8±1.7 to 38.0±4.5 ms (P<0.05, 0 to 20 μM capsaicin). With capsaicin present, pressure

increased $t_{1/2R}$ by 19.2±8.2 ms (P<0.05 to drug with no pressure), whereas shear reduced $t_{1/2R}$ in whole cells by 9.5±0.9 ms (P<0.05 to drug with no shear). These data show that shear stress on whole cells and pressure on patches had opposite effects (shear accelerating, suction slowing). In both approaches, the recovery from inactivation was delayed by capsaicin, and capsaicin further delayed recovery in patches but accelerated recovery in whole cells.

Effects of capsaicin and mechanical stimuli on use-dependent inactivation

Capsaicin can stabilize the inactivated state of $Na_V1.5$ through use-dependent inhibition.[25] The effects of pressure on use-dependent $Na_V1.5$ function have not been fully explored, and we tested whether force can alter use-dependent inactivation of $Na_V1.5$ in the absence or presence of capsaicin (Figure 4A-F). To measure the use-dependent inhibition of $Na_V1.5$ expressed in HEK cells, Na^+ currents elicited by steps to 0 or -30 mV in patches or whole cells were sampled at 3-33 Hz or 0.3-50 Hz, resepectively. Without force or drug, the maximum use-dependent inhibition of $Na_V1.5$ was 80.9±7.9% with a half-frequency ($f_{1/2}$) of 22.3±2.6 Hz in patches (Table 2, Figure 4C,E-F), and 64.6±2.4% with a $f_{1/2}$ of 26.1±2.0 Hz in whole-cells (Table 3, Figure 4D-F). The use dependence did not change with either pressure or shear in the absence of capsaicin (P>0.05 to no force, Tables 2-3, Figure 4C-F). In the absence of shear, capsaicin increased the maximum use-dependent inhibition of $Na_V1.5$ to 89.0±1.4% and decreased $f_{1/2}$ to 18.9±1.0 Hz (P<0.05, 0 to 20 μM capsaicin). In the presence of capsaicin, shear produced a modest decrease in the maximum use-dependent inhibition (5.1±0.9%; P<0.05 to drug with no shear), and $f_{1/2}$ was unaffected, suggesting that shear partially reverses the use-dependent inhibition of $Na_V1.5$ promoted by capsaicin. In patches, capsaicin affected neither the use-dependent inhibition nor $f_{1/2}$ at rest (P>0.05, 0 to 20 μM capsaicin) but increased the pressure-sensitivity ($f_{1/2}$ decreased by 2.4±3.3 Hz; P<0.05 to drug with no pressure), suggesting that pressure is synergestic with capsaicin to

decrease the frequency at which $Na_V1.5$ undergoes use dependent inhibition. Together, our results suggest that though capsaicin enhances use-dependent inhibition, its effect on force-dependent changes to $Na_V1.5$ use dependence may be specific to the type of force applied.

DISCUSSION

Our study aimed to test the impact of a membrane-permeable amphipathic agent on $Na_V1.5$ mechanosensitivity. We selected compounds with high partition coefficients and tested the inhibition of $Na_V1.5$ voltage-gating. Capsaicin inhibited $Na_V1.5$ mechanosensitivity comparable to the amphipaths lidocaine[13, 14] and ranolazine[14]. Capsaicin consistently inhibited the effects of pressure or shear stress on $Na_V1.5$ in membrane patches or whole cells, respectively, by (1) diminishing the mechanosensitive increases in Na^+ current, (2) shifts in steady-state voltage dependence, and (3) acceleration of $Na_V1.5$ gating kinetics.

Quantifying mechanosensitive changes in ion channel function can be challenging, and few studies have explored $Na_V1.5$ mechanosensitivity using whole-cell and patch modes in parallel.[1, 11, 13] To our knowledge, this is the first study to compare the effects of pressure and shear on the mechanosensitive operation of $Na_V1.5$ in the absence nor presence of drug. Most of $Na_V1.5$'s mechanosensitive responses and capsaicin's effects on $Na_V1.5$ MS were similar in the two testing modes. Both produced an increase in peak Na^+ current, a shift in $V_{1/2A}$, a shift in $V_{1/2I}$, and an acceleration in τ_A. Capsaicin, using either approach, inhibited MS effects on the above biophysical parameters. This is important because mechanical strain leads to faster and greater Na^+ influx, which increases Na_V channel availability and further depolarizes the membrane (closer to the threshold to fire action potentials or elicit autonomous membrane depolarizations). Capsaicin would reduce Na^+ influx, slow membrane depolarization (or hyperpolarize the membrane) thereby reducing the effect of mechanical forces on Na_V channel availability.

Surprisingly, we found opposite responses in the pressure- and shear-sensitivity of $Na_V1.5$ inactivation recovery and use-dependent inactivation. Pressure increased the inactivation recovery time, whereas shear stress decreased it. Addition of capsaicin to patches resulted in a

299 further delay of Nav1.5 inactivation recovery in patches under pressure, in contrast to whole
300 cells, where shear stress accelerated inactivation recovery whether or not capsaicin was present.
301 Similarly, capsaicin and pressure, when applied together, decreased the frequency of use-
302 dependent inactivation in patches, while in whole cells capsaicin alone lowered use-dependence
303 frequency, a process unaffected by shear stress. Pressure has previously been shown to prolong
304 Nav1.5 inactivation recovery time in patches[1], but the effect of shear stress on inactivation
305 recovery or use-dependent inhibition of Nav1.5 was previously unknown. The opposite
306 responses in use dependence and recovery using the two approaches are independent of
307 capsaicin, and therefore suggest that these mechanical stimuli may act through different
308 mechanisms.[21, 26] Conceivably, the effect of pressure or shear stress on the membrane or
309 cytoskeleton could be different. The similar increases in peak current, as a result of pressure or
310 shear, reflect a greater probability of Nav channels in the open state, whereas the divergent
311 effects of pressure and shear on use-dependence and inactivation recovery represent different
312 pathways by which Nav channels are entering or leaving the inactivated state. Shear can lead to
313 uniaxial elastic tension along the membrane, yielding asymmetrical sliding of lipid membrane
314 leaflets[29,30], and the effects of lipid bilayer thinning affect some functional modes, such as
315 inactivation, more than others[27, 28]. Meanwhile, macroscopic patch suction can create unequal
316 transmembrane surface tension[2, 21, 29], with the tension being greatest at the top of the dome.
317 Therefore, while both stimuli may be used to study mechanosensitivity of Nav1.5 and other
318 mechanosensitive ion channels, functional consequences may be dependent on channel's
319 functional modes under study.

320 Capsaicin can inhibit Nav1.5 at rest by promoting Nav channel inactivation but shows
321 greater potency for inhibiting the inactivation-removed (IR) Nav channel triple mutant, WCW, on

the pore lumen DIS6 segment, in the analogous position and across from F1760 in the local anesthetic binding site on the DIVS6 segment.[25, 30] Interestingly, the IR sequence in $Na_V1.5$ (L407W-L409C-A410W) would be 9 amino acids downstream from and functionally similar to IR mutant T220A in bacterial homolog NaChBac, which has greater shear-sensitivity than its wild-type. Whether inhibition of mechanosensitivity by capsaicin requires the LXLA sequence in $Na_V1.5$ remains to be seen, yet finding a shared interaction motif suggests that the LA binding region might sensitize $Na_V1.5$ and other mechanosensitive voltage-gated channels[31] or mechano-gated channels[32] to amphipathic MS inhibition. Capsaicin had divergent effects on pressure- and shear-sensitivity of $Na_V1.5$ use-dependent inhibition or inactivation recovery, suggesting that specific types of force may differentially modulate $Na_V1.5$ mechanosensitivity.

Another amphiphilic $Na_V1.5$ modulator, ranolazine, inhibits the increase in peak Na^+ current and the hyperpolarization of voltage dependence of activation induced by pressure or shear stress comparable to capsaicin[14]. Ranolazine is an anti-ischemic agent that may cause constipation as a common side effect [33]; muscle contractility in human colon smooth muscle cells was lost when ranolazine inhibited $Na_V1.5$ peak current and mechanosensitivity.[34] The effects of capsaicin, lidocaine[13], and ranolazine[14] as mechanosensitivity inhibitors demonstrate that membrane-permeable amphipathic agents may be candidates for modulating $Na_V1.5$ mechanosensitivity and targeting dysfunction in mechanosensitive channelopathies. Amphipathic drugs are widely used in the clinical practice to target ion channels, but are rarely used for mechano-modulation.[12,13] Channelopathies involving mechanosensitive dysfunction are an emerging area of study.[6, 7, 9-11, 35] Ion channelopathies in voltage-sensitive mechano-gated Piezo channels [36-38] and channelopathies affecting $Na_V1.5$ mechanosensitivity have been identified, but both lack treatment options. Hence, drugs that can target and modulate mechanosensitivity carry promise for treating disease.

Capsaicin inhibits its canonical target TRPV1 in sensory neurons to improve GI dysfunction in IBS-D patients[39]; however, TRPV1 is not pressure-sensitive up to -90 mmHg[40] and does not have high expression in HEK cells[29, 41]. Capsaicin has shown promise in targeting pain in IBS. Interestingly, it also has effects on gut motility[42-44], possibly through it's function on $Na_V1.5$ mechanosensivity. Therefore, there may be an exciting possibility of using capsaicin to affect sensory (TRPV1) and motility (SCN5A/$Na_V1.5$) processes by different mechanisms in the GI tract.

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TABLES

Table 1. Partition coefficients and IC_{50} values for amphipathic agents. Partition coefficients denoted logP_{OW} for amiodarone, capsaicin, propranolol and Triton-X100 were previously reported[17-20]. IC_{50}, concentration at which an amphipathic agent inhibited half of the maximum peak whole cell Na^+ current from HEK293 cells transfected with $Na_V1.5$.

	Partition coefficient (logP_{OW})	IC_{50} (μM)	Slope
Amiodarone	7.2	8.4	0.50
Capsaicin	3.04	60	0.64
Propranolol	3.48	7.6	1.01
Triton X-100	4.6	5.3	1.00

Table 2. Effect of capsaicin on pressure-induced $Na_V1.5$ mechanosensitivity in cell-attached patches. Effects of pressure (0 or -30 mmHg) on parameters of macroscopic Na^+ currents without (0 μM) or with capsaicin (20 μM): maximum peak Na^+ currents normalized to controls at 0 mmHg (I_{MAX}), voltage dependence of activation ($V_{1/2A}$) or inactivation ($V_{1/2I}$), time constant of activation (τ_A), time of inactivation recovery ($t_{1/2R}$), slope of inactivation recovery (slope), maximum use-dependent inhibition (block), frequency of use-dependent inhibition ($f_{1/2}$). n = 8-24 cells, *P<0.05, 0 to -30 mmHg or †P<0.05, 0 to 20 μM capsaicin by a 2-way ANOVA with Tukey post-test.

	0 μM				20 μM			
	0 mmHg	-30 mmHg	Change	n	0 mmHg	-30 mmHg	Change	n
I_{MAX} (%)	100.0±0.0	116.6±2.4*	16.6±2.4	24	100.0±0.0	104.8±3.0†	4.8±3.0	14
$V_{1/2A}$ (mV)	-41.9±3.0	-46.4±3.0*	-4.5±0.6	24	-57.5±2.8†	-60.0±3.1*†	-2.4±0.6	14
$V_{1/2I}$ (mV)	-54.1±3.1	-60.1±3.3*	-6.0±0.9	24	-70.5±3.3†	-71.9±3.5†	-1.4±1.2	14
τ_A (ms)	0.32±0.04	0.26±0.04*	-20.0±5.3%	24	0.23±0.04	0.21±0.04	-11.0±5.4%	14
$t_{1/2R}$ (ms)	13.2±2.5	22.1±4.9*	8.9±3.9	11	29.5±6.4†	48.7±9.4*†	19.2±8.6	11
Slope (ms^{-1})	1.3±0.1	1.2±0.1	-0.10±0.17	11	1.9±0.3	2.3±0.8	0.41±0.87	11
Block (%)	80.9±7.9	77.8±8.6	3.2±9.4	11	93.6±4.5	85.5±8.5	4.7±3.8	8
$f_{1/2}$ (Hz)	22.3±2.6	20.0±2.1	-2.4±3.3	11	19.1±2.5	14.8±1.4*	-4.2±1.4	8

Table 3. Effect of capsaicin on shear-induced $Na_V1.5$ mechanosensitivity whole cells. Effects of shear stress (0 or 10 mL/min) on parameters of whole cell Na^+ currents without (0 μM) or with capsaicin (20 μM): maximum peak conductance (G_{MAX}), maximum peak current density (I_{PEAK}), voltage dependence of activation ($V_{1/2A}$) or inactivation ($V_{1/2I}$), time constants of activation (τ_A) and fast (τ_F) or slow inactivation (τ_S), time of inactivation recovery ($t_{1/2R}$), slope of inactivation recovery (slope), maximum use-dependent inhibition (block), frequency of use-dependent inhibition ($f_{1/2}$). n = 8-18 cells, **P*<0.05, 0 to 10 mL/min or †*P*<0.05, 0 to 20 μM capsaicin by a 2-way ANOVA with Tukey post-test.

	0 μM				20 μM			
	0 mL/min	10 mL/min	Change	n	0 mL/min	10 mL/min	Change	n
G_{MAX} (nS)	0.98±0.13	1.24±0.22*	0.26±0.10	12	0.78±0.12†	0.85±0.15†	0.08±0.05	12
I_{PEAK} (pA/pF)	-66.8±12.3	-86.6±17.7*	16.0±3.1%	12	-56.7±58.5†	-58.5±11.6†	3.1±3.8%†	12
$V_{1/2A}$ (mV)	-57.3±1.7	-58.9±1.4*	-1.5±0.6	12	-58.9±1.5†	-59.2±1.6	-0.3±0.1	12
$V_{1/2I}$ (mV)	-90.3±3.3	-92.7±2.6*	-2.5±0.9	12	-95.4±2.6†	-95.8±2.7†	-0.5±0.6	12
τ_A (ms)	0.5±0.05	0.39±0.02*	-20.4±3.3%	12	0.39±0.04†	0.38±0.04	-1.3±7.0%†	12
τ_F (ms)	0.82±0.08	0.57±0.04*	-27.5±4.0%	12	0.80±0.08	0.56±0.05*	-25.1±5.9%	12
τ_S (ms)	4.7±0.3	3.5±0.2*	-24.2±4.4%	12	3.8±0.3†	2.9±0.2*†	-20.5±4.8%	12
$t_{1/2R}$ (ms)	18.8±1.7	16.6±1.8*	-2.2±0.6	8	38.0±4.5†	28.4±4.2*†	-9.5±0.9†	8
slope (ms^{-1})	-1.26±0.04	-1.28±0.05	-0.02±0.02	8	-1.43±0.02†	-1.42±0.06	0.01±0.04	8
Block (%)	64.6±2.4	64.7±2.6	0.2±1.7	18	89.0±1.4†	83.9±2.0*†	-5.1±0.9†	10
$f_{1/2}$ (Hz)	26.1±2.0	27.3±2.3	1.2±0.9	18	18.9±1.0†	21.0±1.7	2.1±1.4	10

FIGURE LEGENDS

Figure 1. Amphipathic compounds inhibit voltage-gated Na^+ currents from $Na_V1.5$ channels expressed in HEK293 cells. *A*, Molecular structures of the amphipaths (from *left* to *right*): amiodarone, capsaicin, propranolol, and Triton X-100 .*B-C*, Representative Na^+ currents elicited by a step from -120 to the -35-mV test voltage (*B*), and peak Na^+ current-voltage plots across all test voltages (*C*) with 10^{-9} to 10^{-4} M (*blue-red spectrum*) of membrane-permeable amphipathic compounds in the extracellular solution . *D*, Dose-response curves for maximum peak Na^+ current of $Na_V1.5$ *vs.* amphipathic concentration; IC_{50} values: amiodarone, 8.4 μM; capsaicin, 60.2 μM; propranolol, 7.6 μM; Triton X-100, 5.3 μM.

Figure 2. Capsaicin inhibits pressure- and shear-sensitivity of $Na_V1.5$. *A*, Representative $Na_V1.5$ currents elicited by voltage ladders ranging -100 to 0 mV in a cell-attached patch (*A*) or -120 mV to -30 mV in a whole cell (*B*), recorded at rest (*filled symbols*) or with force (*empty symbols*), in the presence of 0 μM (*black*) or 20 μM capsaicin (*red*). Difference currents were constructed by subtracting the control Na^+ currents from the pressure- (*A*) or shear-stimulated (*B*) currents. *C-D*, Steady-state activation (*C*) and inactivation (*D*) curves of Na^+ currents in cell-attached patches (*left*) or whole cells (*right*), recorded at rest (*filled symbols*) or with force (*empty symbols*), in the presence of 0 μM (*black*) or 20 μM capsaicin (*red*). *E-H*, Maximum peak Na^+ current (*E*), time constant of activation (*F*), and voltage dependence of activation (*G*, $V_{1/2A}$) or inactivation (*H*, $V_{1/2I}$), recorded with 0 or -30 mmHg pressure in the patch (*left*) and 0 or 10 mL/min shear stress in whole cells (*right*) in the presence of 0 μM (*black*) or 20 μM capsaicin (*red*). n = 12-24 cells, **P*<0.05 comparing 0 to -30 mmHg or 0 to 10 mL/min, †*P*<0.05 comparing 0 to 20 μM capsaicin by a 2-way ANOVA with Tukey post-test.

Figure 3. Effects of capsaicin on mechanosensitivity of $Na_V1.5$ inactivation recovery time. *A-B,* Representative $Na_V1.5$ currents at -20 mV in a cell-attached patch (*A*, ●) or -30 mV in a whole cell (*B*, ■), elicited after recovering from the control pulse for 3-300 ms at -120 mV (*A*) or 3-1000 ms at -130 mV (*B*). Na^+ currents were recorded at rest (*grey*) or with force (*dark traces*: *A*, -30 mmHg pressure; *B*, 10 mL/min shear stress) in the presence of 0 μM (*top*) or 20 μM capsaicin (*bottom*). *C-D,* Normalized peak Na^+ current versus recovery time in the presence of 0 μM (*black*) or 20 μM capsaicin (*red*), at 0 (●) or -30 mmHg pressure (○) in the patch (*C*) or at 0 (■) or 10 mL/min (□) shear stress in whole cells (*D*). *E-F*, Inactivation recovery times ($t_{1/2}$) versus 0 or -30 mmHg pressure in the patch (*E*) and 0 or 10 mL/min shear stress in whole cells (*F*) with 0 μM (*black*) or 20 μM capsaicin (*red*). n = 8-11 cells, **P*<0.05 comparing 0 to -30 mmHg or 0 to 10 mL/min, †*P*<0.05 comparing 0 to 20 μM capsaicin by a 2-way ANOVA with Tukey post-test.

Figure 4. Effects of capsaicin on mechanosensitivity of $Na_V1.5$ use-dependent inactivation. *A-B,* Representative $Na_V1.5$ currents at the 20^{th} pulse to -20 mV in a cell-attached patch (*A*, ●) or to -40 mV in a whole cell (*B*, ■), elicited at interpulse frequencies 3-33 Hz (*A*) or 3-50 Hz (*B*). Na^+ currents were recorded at rest (*grey*) or with force (*dark traces*: *A*, -30 mmHg pressure; *B*, 10 mL/min shear stress) in the presence of 0 μM (*top*) or 20 μM capsaicin (*bottom*). *C-D,* Use-dependent inhibition of peak Na^+ current versus interpulse frequency in the presence of 0 μM (*black*) or 20 μM capsaicin (*red*), at 0 (●) or -30 mmHg pressure (○) in the patch (*C*) or at 0 (■) or 10 mL/min (□) shear stress in whole cells (*D*). *E-F*, Maximum use-dependent inhibition (*E*) or frequency of use-dependent inhibition (*F*) versus pressure in the patch (*left*) and shear stress in whole cells (*right*) with 0 μM (*black*) or 20 μM capsaicin (*red*). n = 8-18 cells, **P*<0.05 comparing

0 to -30 mmHg or 0 to 10 mL/min, †$P<0.05$ comparing 0 to 20 μM capsaicin by a 2-way ANOVA with Tukey post-test.

FIGURES

Figure 1.

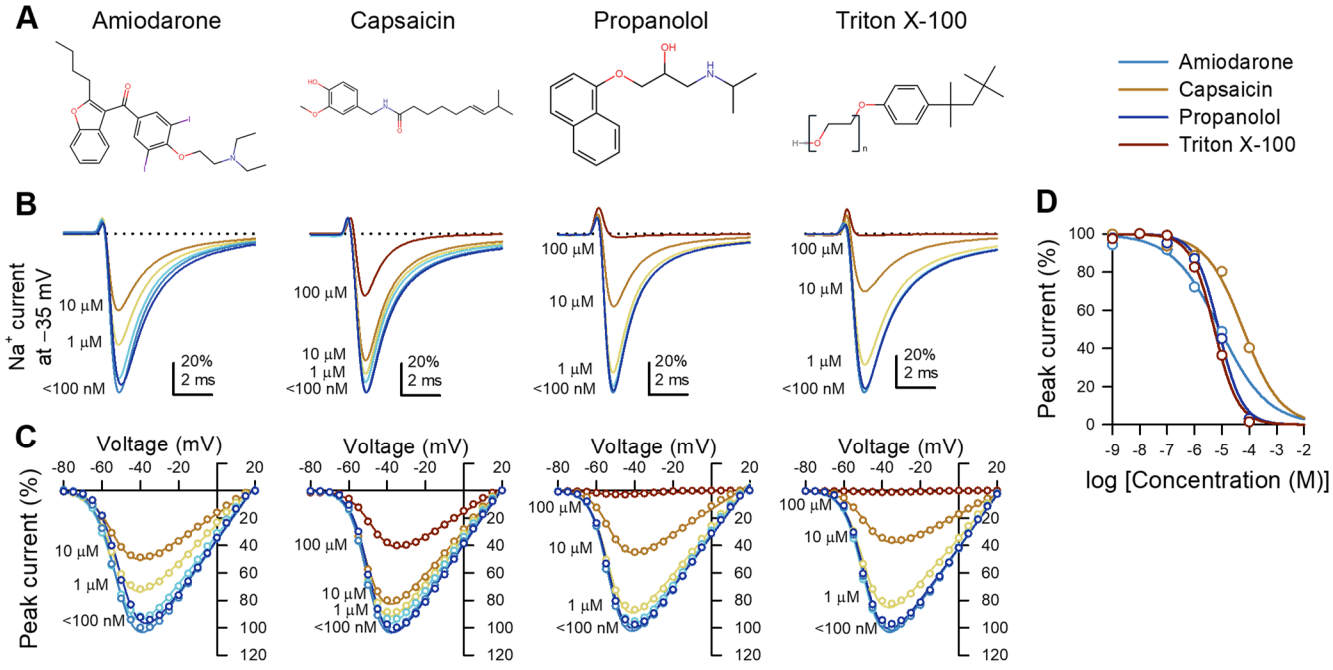
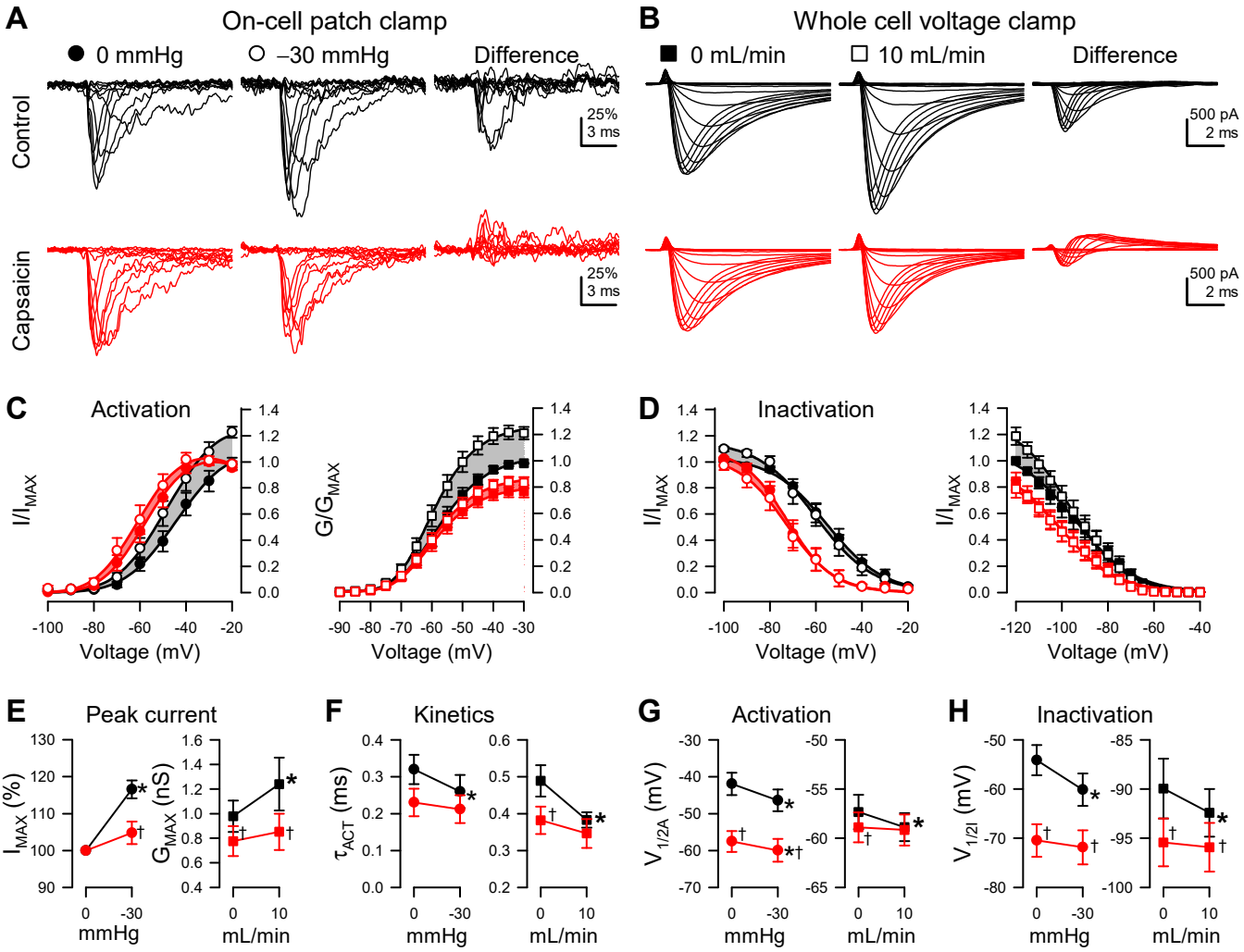
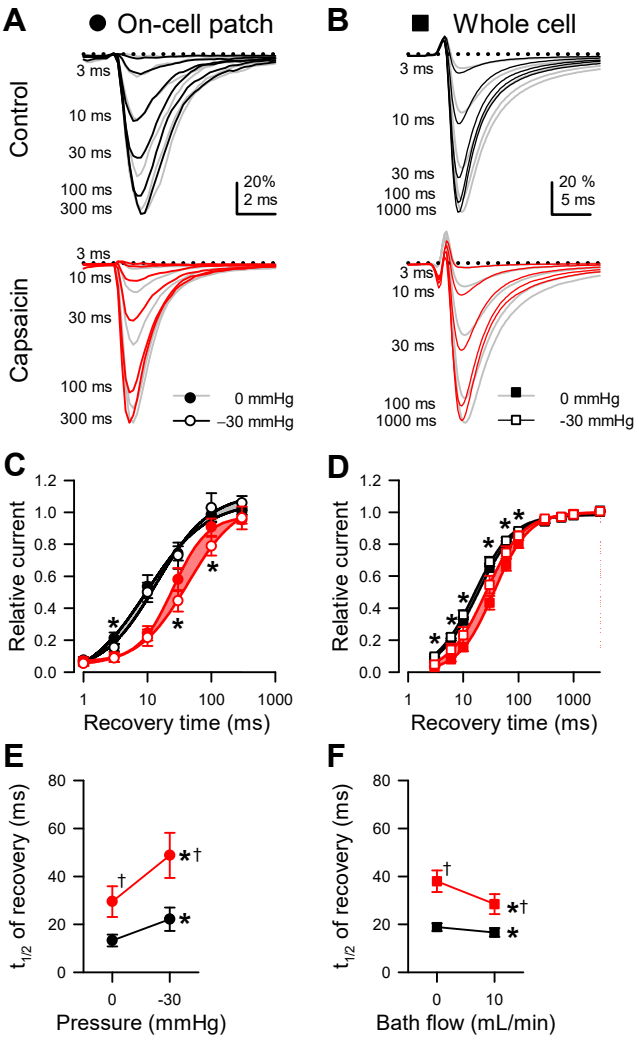


Figure 2.



572 **Figure 3.**



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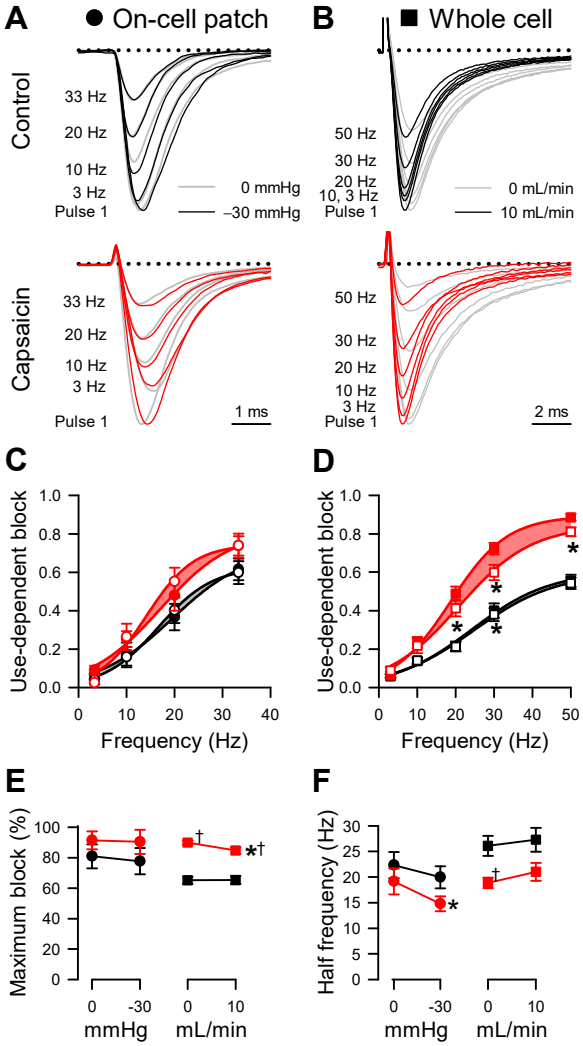
574 **Figure 4.**



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