**Rapid Communication**

**Na⁺-Gated Nonselective Cation Channel From Lobster Olfactory Projection Neurons**

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Zhainazarov, Aslbek B. and Barry W. Ache. Na⁺-gated nonselective cation channel from lobster olfactory projection neurons. J. Neurophysiol. 80: 3387–3391, 1998. A nonselective cation channel specifically activated by intracellular Na⁺ was identified in cell-free patches taken from cultured lobster olfactory projection neurons. Na⁺ reversibly activates the channel in a concentration-dependent manner, with a "half-effect" Na⁺ concentration of 76.4 mM at −60 mV. The conductance of the channel is 32 pS. The channel is permeable to both alkali metal (Li⁺ > Na⁺ > K⁺ > Rb⁺ > Cs⁺) and divalent (Ca²⁺ > Mn²⁺ > Sr²⁺ > Ba²⁺ > Ba²⁺ > Na⁺) cations. The presence of a channel with the ability to generate plateau potentials suggests that the channel may potentially contribute to oscillatory behavior in these olfactory interneurons.

**Methods**

Neurons were obtained from either the lateral (LC) or medial (MC) soma clusters in the deutocerebrum of the Caribbean spiny lobster (Panulirus argus). The LC contains exclusively somata of olfactory projection neurons, whereas the MC contains exclusively somata of olfactory local interneurons (Sandeman et al. 1992). Somata from the LC and the MC were isolated and maintained in primary culture as described previously (Zhainazarov et al. 1998). Inside-out patch-clamp recordings were performed as described by Zhainazarov and Ache (1997). Excised patches were perfused using a rapid solution changer with nine delivery ports (RSC-100; Bio-Logic, Claix, France) as described previously (Zhainazarov and Ache 1995). Single-channel currents were recorded with a patch-clamp amplifier (Axopatch 200A; Axon Instruments, Foster City, CA), low-pass filtered at 1 kHz (−3 dB; 4-pole Bessel filter), digitized at 10 kHz by a personal computer with an A/D, D/A interface (TL-1; Axon Instruments) using pClamp software (pClamp 6.0; Axon Instruments), and stored on the computers’ hard disk for later analysis.

Panulirus saline (PS) consisted of (in mM) 458 NaCl, 13.4 KCl, 13.4 Na₂SO₄, 13.6 CaCl₂, 9.8 MgCl₂, 2 glucose, and 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH 7.4 adjusted with NaOH. Unless noted otherwise, the patch pipette was filled with PS solution. Sodium-free solution consisted of (in mM) 210 LiCl, 11 ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA), 1 CaCl₂, 696 glucose, and 10 HEPES, pH 7.4 adjusted with Tris base. The free calcium concentration was estimated using Chelator software (Schoenmakers et al. 1992) at ~10 nM. In some experiments, part of the LiCl in the sodium-free solution was substituted with an equivalent concentration of chloride salts of other alkali metal cations (NaCl, KCl, CsCl, and RbCl). To measure the extent of divalent cation permeation through the channel, the patch pipette was filled with (in mM) 10 HEPES, and 105 chloride of one of the following divalent cations: Ca²⁺, Mg²⁺, Ba²⁺, Sr²⁺, and Mn²⁺. pH 7.4 adjusted with Tris base.

**Results**

Na⁺-gated channel activity in patches excised from central olfactory neurons

Inside-out patches showed unitary currents that were reversibly activated by Na⁺ applied to the intracellular face of the patch (Fig. 1). Such activity occurred in 41% of 91...
patches taken from LC neurons, and 25% of 65 patches taken from MC neurons. Li\(^+\), K\(^+\), Cs\(^+\), and Rb\(^+\) failed to activate the channel, indicating that the effect was specific to Na\(^+\) (not shown). In all intracellular solutions the free Ca\(^{2+}\) concentration was held at 10 nM by buffering with 11 mM EGTA. In the absence of Na\(^+\), intracellular Ca\(^{2+}\) (1–200 \(\mu M\)) failed to activate the channel (not shown). However, applying 10 nM to 2 mM Ca\(^{2+}\) in the presence of 90 mM Na\(^+\) to the intracellular face of inside-out patches reversibly reduced channel activity starting at 10 \(\mu M\) (not shown). These three lines of evidence exclude the possibility that the channel was being activated by Ca\(^{2+}\). The majority of the patches contained multiple Na\(^+\)-gated nonselective cation channels (range 1–10, mean 3), as judged by counting multiple openings at high Na\(^+\) concentration. Three patches contained only a single Na\(^+\)-gated channel. The data described below were all collected from LC neurons, although the similar results (not shown) were obtained for MC neurons.

**Current-voltage (I-V) relationship of the unitary current**

The I-V relationship of the channel was measured in inside-out patches with the patch pipette filled with PS and [Na\(^+\)], was 210 mM. Figure 2A illustrates examples of unitary currents recorded at various membrane potentials from a patch containing two Na\(^+\)-gated channels. Figure 2B shows 30 superimposed current traces recorded from the same patch in response to a voltage ramp from −100 to +100 mV (350 ms). The single-channel current reversed at 28.4 ± 1.8 (SE) mV (n = 3; Fig. 2B). The I-V relationship of the channel at negative potentials (−100 to −20 mV) had a slope conductance of 32.4 ± 1.5 pS (Fig. 2C).

**Dose dependency of Na\(^+\) activation**

Solutions containing different concentrations of Na\(^+\) were applied to the intracellular side of inside-out patches. Figure 2D illustrates examples of channel activity at five different concentrations. Channel openings were absent in Na\(^+\)-free solution but appeared in solutions containing 10 mM Na\(^+\) and increased in frequency at higher [Na\(^+\)] (Fig. 2D). When [Na\(^+\)] was increased from 10 to 210 mM, the open probability of the channel (\(P_o\)) increased from 0.004 ± 0.002 to 0.83 ± 0.03 (n = 3) at −60 mV. The open probability of the channel, plotted as a function of [Na\(^+\)], could be fitted by the Hill equation with parameters of \(P_o,max = 0.92 ± 0.05, EC_{50} = 76.4 ± 4.8 \text{ mM and } m = 2.9 ± 0.5 \) (solid line, Fig. 2E).

**Ionic selectivity of the channel**

To determine ionic selectivity of the channel among alkali metal cations, we perfused the intracellular face of the inside-out patch with 90 mM Na\(^+\) + 120 mM of one of the following monovalent cations: Na\(^+\), Li\(^+\), K\(^+\), Cs\(^+\), and Rb\(^+\). The patch pipette contained 210 mM Na\(^+\). Under these ionic conditions, the reversal potential (\(E_r\)) of the Na\(^+\)-gated channel current measured by applying voltage ramps (−100 to +100 mV; 350 ms) was 0.1 ± 0.1 for Na\(^+\), −3.2 ± 0.3 for Li\(^+\), 5.5 ± 0.2 for K\(^+\), 12.4 ± 1.1 for Cs\(^+\), and 8.9 ± 0.6 for Rb\(^+\) (Fig. 3A; n = 3–10). The permeability of ion \(X^+\) relative to Na\(^+\) was calculated from the shift of the \(E_r\) by using the Goldman-Hodgkin-Katz equation: 

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E_r = (RT/F) \ln \left( \frac{P_{X^n}Na^+}{P_{Na^n}X^n} \right)
\]

where \(P_{X^n}Na^+\) and \(P_{Na^n}X^n\) have their usual significance. The permeability ratio sequence (\(P_{X^n}/P_{Na^n}\)) was Li\(^+\) (1.24) > Na\(^+\) (1.00) > K\(^+\) (0.66) > Rb\(^+\) (0.48) > Cs\(^+\) (0.32), indicating that the channel is fairly nonselective among alkali metal cations. We also determined the ionic selectivity of the channel for divalent cations using inside-out patches recorded with pipettes filled with 105 mM chlorides of one of the following divalent cations: Ba\(^{2+}\), Mn\(^{2+}\), Mg\(^{2+}\), Ca\(^{2+}\), Sr\(^{2+}\). The intracellular face of the patches were bathed in 210 mM Na\(^+\). The mean reversal potential was 55.8 ± 1.6 for Ca\(^{2+}\), 32.2 ± 1.7 for Mg\(^{2+}\), 36.0 ± 0.2 for Ba\(^{2+}\), 48.5 ± 3.6 for Mn\(^^{2+}\), and 46.0 ± 0.7 for Sr\(^{2+}\), respectively (Fig. 3B; n = 3–6). Estimation of the permeability of the divalent cation \(Y^{2+}\) relative to Na\(^+\) was based on the value of \(E_r\) and calculated by using the following equation (Fatt and Ginsborg 1958): 

\[
P_{Y^{2+}}/P_{Na^{+}} = \exp(E_r/RT) (\exp(E_r/RT + 1)/4, where \{Y^{2+}\} is the extracellular concentration of \(Y^{2+}\). The \(P_{Y^{2+}}/P_{Na^{+}} sequence for the divalent cations was Ca\(^{2+}\) (45.7) > Mn\(^{2+}\) (26.5) > Sr\(^{2+}\) (22.0) > Mg\(^{2+}\) (10.7) > Ba\(^{2+}\) (8.2) > Na\(^+\) (1.0), indicating that the channel is much more permeable to divalent cations than to Na\(^+\).
FIG. 2. Current-voltage relationship and dose-response curve for the Na$^+$-gated channel. A: current records of Na$^+$-gated channel activity at various membrane potentials with the pipette filled with PS. Patch contained 2 Na$^+$-gated channels. B: 30 superimposed single-channel current records (bottom trace) evoked by a voltage ramp (top trace). $E_x$ is indicated by vertical dashed line. C: plot of current-voltage relationship of the Na$^+$-gated channel under these conditions. Solid line, linear regression through the data. D: traces of Na$^+$-gated channel activity at the indicated [Na$^+$]$_i$. Part of the LiCl in the sodium-free solution was substituted with an equivalent concentration of NaCl. Membrane potential, $-60$ mV. E: plot of channel open probability plotted as a function of [Na$^+$]. Solid curve, fit of the Hill equation to the data. In C and E, points given as means $\pm$ SD of 3 experiments. In A, B, and D, traces obtained from the same inside-out patch. In A and B, bath solution contained 210 mM NaCl. Baseline depicted by dashed line. In A–E, the patch pipette was filled with PS.

**DISCUSSION**

The ability of Na$^+$ to specifically and reversibly activate unitary currents in a dose-dependent manner when applied to the inner face of membrane patches from the soma of the cultured olfactory projection neurons (and local interneurons) provides strong evidence that these cells express a Na$^+$-gated channel. The main properties (single-channel...
conductance, sensitivity to intracellular Na\(^+\), and ionic selectivity) of the Na\(^+\)-gated channel in the CNS are similar to those of the Na\(^+\)-gated nonselective cation channel described previously in the outer dendrite of the olfactory receptor neurons (Zhainazarov and Ache 1997): 1) the central channel has a slope conductance of 32.4 versus 25.7 pS for the peripheral channel; 2) both channels are relatively nonselective among monovalent cations (Na\(^+\), Li\(^+\), K\(^+\), Cs\(^+\), and Rb\(^+\)) and highly permeable to divalent cations \((P_{Ca}/P_{Na}: 45.7 \text{ for the central channel vs. } 39.0 \text{ for the peripheral channel}); 3) for both these channels the dependence of the open probability on \([\text{Na}^+]_i\) is sigmoidal, indicating that more than one Na\(^+\) must bind to activate the channel. Thus the lobster Na\(^+\)-gated nonselective cation channel does not appear to be confined to primary olfactory receptor neurons and may serve a more general physiological role.

The functional significance of the channel in the lobster CNS remains to be determined, however. Membrane proteins could readily redistribute as the cells reorganize in culture, and the channel would not necessarily have to function in the soma where it was recorded. The dendritic terminals of these cells are extremely fine, spatially restricted compartments (Schmidt and Ache 1996), not unlike the outer dendrites of the primary receptor cells where odor-activated Na\(^+\)/influx appears to be sufficient to activate the nonselective among monovalent cations (Na\(^+\), Li\(^+\), K\(^+\), Cs\(^+\), and Rb\(^+\)) and highly permeable to divalent cations channel. Assuming that the Na\(^+\)-gated channel also occurs in the dendritic compartment of the central neurons, and that the plasma concentration of ions approximates that of the seawater surrounding the outer dendrites of the receptor cells (Gleeson et al. 1993), it is reasonable to hypothesize that the central channel could be activated by Na\(^+\)/influx through Na\(^+\)/permeant ion channels.

Once activated, the noninactivating Na\(^+\)-gated channel presumably would manifest itself as a slow inward current.
Slow inward currents, or plateau potentials, occur in a wide variety of vertebrate and invertebrate neurons (Hartline and Graubard 1992). The ionic mechanisms underlying plateau potentials have been ascribed to activation of a persistent voltage-gated Na⁺ conductance, a low-threshold voltage-gated Ca²⁺ conductance, and a Ca²⁺-activated nonselective cation conductance (Llinás 1988; Swandulla and Lux 1985; Taylor 1993). We propose, therefore, that the Na⁺-gated nonselective cation conductance could also generate prolonged, slow regenerative depolarizations (plateau potentials). Plateau potentials endow cells with switchlike quasistable operating characteristics and play a crucial role in generating rhythmic oscillations of the membrane potential (Hartline and Graubard 1992). Odors evoke rhythmic oscillations in neurons at the first synaptic level of the olfactory pathway in many animals, including crustaceans (review: Laurent 1996), where they are suspected to play an essential role in encoding the temporal features of odor representation (Laurent 1997).

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