Local Anesthetic-like Inhibition of Voltage-gated Na\textsuperscript{+} Channels by the Partial \(\mu\)-opioid Receptor Agonist Buprenorphine

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ABSTRACT

Background: Opioids induce analgesia mainly by inhibiting synaptic transmission via G protein-coupled opioid receptors. In addition to analgesia, buprenorphine induces a pronounced antihyperalgesia and is an effective adjuvant to local anesthetics. These properties only partially apply to other opioids, and thus targets other than opioid receptors are likely to be employed. Here we asked if buprenorphine inhibits voltage-gated Na\textsuperscript{+} channels.

Methods: Na\textsuperscript{+} currents were examined by whole cell patch clamp recordings on different recombinant Na\textsuperscript{+} channel \(\alpha\)-subunits. The effect of buprenorphine on unmyelinated mouse C-fibers was examined with the skin-nerve preparation. Data are presented as mean \pm SEM.

Results: Buprenorphine induced a concentration-dependent tonic (IC\textsubscript{50} 33 \pm 2 \mu M) and use-dependent block of endogenous Na\textsuperscript{+} channels in ND7/23 cells. This block was state-dependent and displayed slow on and off characteristics. The effect of buprenorphine was reduced on local anesthetic insensitive Nav1.4-mutant constructs and was more pronounced on the inactivation-deficient Nav1.4-WCW mutant. Neuronal (Nav1.3, Nav1.7, and Nav1.8), cardiac (Nav1.5), and skeletal muscle (Nav1.4) \(\alpha\)-subunits displayed small differences in tonic block, but similar degrees of use-dependent block. According to our patch clamp data, buprenorphine blocked electrically evoked action potentials in C-fiber nerve terminals. Buprenorphine was more potent than other opioids, including morphine (IC\textsubscript{50} 378 \pm 20 \mu M), fentanyl (IC\textsubscript{50} 95 \pm 5 \mu M), sufentanil (IC\textsubscript{50} 111 \pm 6 \mu M), remifentanil (IC\textsubscript{50} 612 \pm 17 \mu M), and tramadol (IC\textsubscript{50} 194 \pm 9 \mu M).

Conclusions: Buprenorphine is a potent local anesthetic and blocks voltage-gated Na\textsuperscript{+} channels via the local anesthetic binding site. This property is likely to be relevant when buprenorphine is used for pain treatment and for local anesthesia.

OPIOIDS are potent analgesics and are frequently employed for treatment of moderate to severe pain. Most opioid-induced effects are mediated by G protein-coupled opioid receptors (OR), which are widely expressed within the peripheral and central nervous systems.\textsuperscript{1,2} Potent opioid-induced analgesia requires expression of the \(\mu\)-OR.\textsuperscript{3,4} However, both \(\delta\)-ORs and \(\kappa\)-ORs also significantly contribute to the antinociceptive properties of opioids.\textsuperscript{5,6} ORs mediate analgesia primarily by inhibiting synaptic transmission in the spinal cord, and this process involves the inhibition of presynaptic Ca\textsuperscript{2+} channels and an activation of postsynaptic K\textsuperscript{+} channels.\textsuperscript{7,8} Despite their high analgesic potency, most opioids do not prevent but rather promote the development of hyperalgesia in rodent and human pain models.\textsuperscript{9,10} The partial OR-agonist buprenorphine makes an exception in this regard, as it induces a pronounced and long-lasting an-
The pharmacology of buprenorphine is rather complex as it acts as a partial µ-OR agonist, a κ-OR antagonist, and a full ORL1 agonist. Although buprenorphine-induced analgesia in rodents requires the µ-OR, it is possible that antihyperalgesia is accomplished by incoherent effects on several OR-subtypes. Activation of ORL1 is likely to be pronociceptive, and it was even suggested that this property compromises the analgesic efficacy of buprenorphine. Supraspinal κ-ORs can mediate hyperalgesia, and therefore an antagonistic action of buprenorphine on κ-ORs could indeed counteract hyperalgesia. However, taken the fact that other opioids have less antihyperalgesic effects as compared with buprenorphine, it seems unlikely that ORs are the sole molecular mechanisms for the induction of antihyperalgesia. Consequently, OR-independent targets of buprenorphine must be taken into account. Interestingly, high concentrations of intrathecally applied buprenorphine were reported to block C- and Aβ-fiber mediated input to the dorsal horn by OR-independent mechanisms. Furthermore, peripherally applied buprenorphine alone or as an adjuvant to local anesthetics (LA) has been reported to induce effective and prolonged pain relief. Thus there are some indications from previous reports for a direct and non-OR mediated effect on neuronal excitability by buprenorphine. In this regard we also found it intriguing that the LA lidocaine induces antihyperalgesia in the same pain model in which the antihyperalgesic property of buprenorphine was observed. Lidocaine is a classic LA and inhibits voltage-gated Na⁺ channels by interacting with the intracellular LA-binding site.

In this study we asked if buprenorphine inhibits or modulates Na⁺ channels in a manner that could counteract the development of hyperalgesia. To address this question we investigated recombinant wild-type and mutant neuronal and nonneuronal α-subunits of Na⁺ channels by conventional patch clamp recordings. Furthermore, the effect of buprenorphine on the excitability of intact C-fibers was investigated by an in vitro skin-nerve preparation.

**Materials and Methods**

**Stable Cell Lines and Transient Transfection**

The dorsal root ganglion neuroblastoma hybridoma cell line ND7/23 (purchased from European Collection of Cell Cultures, Porton Down, United Kingdom) and human embryonic kidney 293t (HEK293t) cells were cultured in Dulbecco’s modified Eagle medium, supplemented with 100 U/ml penicillin/streptomycin, 25 mM HEPES, 10% heat-inactivated fetal bovine serum (all DMEM, Gibco-Invitrogen, Karlsruhe, Germany) and 3 mM taurine (Sigma–Aldrich, Taufkirchen, Germany) at 37°C and 5% CO₂. Transient transfections were carried out as described previously. Stable HEK-Nav1.3 cells (from Professor Stephen Waxman M.D., Ph.D., Department of Neurology, Yale University, New Haven, Connecticut) were used for experiments with out any transfection procedures.

**Whole Cell Patch Clamp Recordings**

Na⁺ currents were recorded with the whole cell configuration of the patch-clamp technique and an Axopatch 200B patch-clamp amplifier (Molecular Devices, Sunnyvale, CA), as described previously. Patch pipettes fabricated from borosilicate glass tubes (TW150F-3; World Precision Instruments, Berlin, Germany) were pulled to a resistance of 1.0–1.9 MΩ after heat-polishing. Currents were filtered at 5 kHz, and sampled at 20 kHz. The series-resistance was compensated by 60–80% to minimize voltage errors and the capacitance artifact was canceled using the amplifier circuitry. Linear leak subtraction, based on resistance estimates from four hyperpolarizing pulses applied before the test pulse, was used for all voltage-clamping recordings, except for the experiments on use-dependent block.

Experiments on HEK293t cells and ND7/23 cells were performed with an external solution containing 65 mM NaCl, 85 mM choline Cl, 2 mM CaCl₂, and 10 mM HEPES (adjusted to pH 7.4 with tetramethylammonium hydroxide) and a pipette solution containing 100 mM NaF, 30 mM NaCl, 10 mM EGTA, and 10 mM HEPES (adjusted to pH 7.2 with CsOH). The reversed Na⁺ gradient was used to minimize the series resistance artifact, which is less serious with outward currents. For experiments on DRG neurons, the extracellular solution contained: 40 mM NaCl, 100 mM choline Cl, 3 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂ and 10 mM Heps. The pipette solution contained 140 mM CsF, 10 mM NaCl, 1 mM EGTA, and 10 mM Heps (adjusted to pH 7.4 with tetramethylammonium hydroxide). The osmolarity of all solutions was adjusted to 300–310 mOsm.

**Single-fiber Recordings**

The isolated skin-saphenous nerve preparation and single-fiber recording technique were performed on adult mice as described elsewhere. Receptive fields of identified single C-fibers were searched by mechanical probing, further characterized with gravity-driven von Frey type pens and with respect to thermal responsiveness. To examine conduction block by buprenorphine, receptive fields were electrically stimulated with twice electrical threshold strength at 0.1 Hz by using a high-impedence needle electrode with blank tip and a constant current stimulus isolator (World Precision Instruments). Buprenorphine was applied inside the metal ring at increasing concentrations from 20, 40, 60, 100, 200, and up to 300 µM each in combination with 10 µM naloxone at intervals of 15 min until the fibers were blocked. Evoked action potentials were recorded from electrically isolated teased single-fibers via gold wire electrodes in an adjacent chamber overlaid with paraffin oil.

**Culture of Dorsal Root Ganglion Neurons (DRG)**

Adult mice of the C57/B16 strain were killed by carbonic acid gas inhalation. DRGs from all spinal levels were removed and cultured as described previously. Recordings were made after approximately 24 h in culture.
Solutions and Chemicals

Morphine, tramadol, fentanyl, sufentanil, buprenorphine, naloxone (Sigma-Aldrich), and remifentanil (Ultiva®, GlaxoSmithKline, Munich, Germany) were either prepared as 10–100 mM stock solutions in Dimethyl sulfoxide or directly prepared in the standard extracellular solution. A 10 mM stock solution of tetrodotoxin (Alomone Labs, Jerusalem, Israel, dissolved in water) was diluted to a working concentration of 300 nM for recordings on Nav1.8. Solutions were applied through a homemade gravity-driven polytetrafluorethylene-glass multiple-barrel perfusion system.

Data Analysis

Patch clamp data were analyzed with the Clampex 8.0 software package (Axon Instruments, Union City, NJ) and single-fiber recordings were analyzed with the software packages DAPSYS (Brian Turnquist, Bethel University, St. Paul, Minnesota) and CED Spike2 (Cambridge Electronic Design, Cambridge, United Kingdom). Curve fitting and statistical analyses were performed with Origin 6.0 (Microcal Software, Northampton, MA). Unless otherwise noted, statistical significance was determined at P < 0.05 and calculated with the two-tailed Student t test. Data are presented as mean ± SEM or fitted value ± SE of the fit. To obtain activation curves, the peak conductance $g_m$ was estimated from the equation $g_m = I/V_{rev}(E_m - E_{rev})$, where $I_{Na}$ is the peak current, $E_m$ is the corresponding voltage, and $E_{rev}$ is the estimated reversal potential. The data were fitted with the Boltzmann equation $g_m/g_{max} = 1/(1 + \exp((E_m - E_0.5)/k_h))$, where $g_{max}$ is the maximum conductance, $E_0.5$ is the voltage at which $g/g_{max} = 0.5$, and $k_h$ is the slope factor. To obtain the inactivation curves, peak current amplitudes evoked by a test pulse were measured, normalized, and plotted against the conditioning prepulse potential. The data were fitted by the Boltzmann equation $y = 1/(1 + \exp(E_{pp} - h_0.5)/k_i)$, where $h_0.5$ is the voltage at which $y = 0.5$ and $k_i$ is the slope factor. To obtain IC$_{50}$ values, peak currents amplitudes at different drug concentrations were normalized to the value obtained in control solution. The data were fitted with the Hill equation $y = y_{max} \times (IC_{50}^n/(IC_{50}^n + C^n))$, where $y_{max}$ is the maximal amplitude, $IC_{50}$ the concentration at which $y/y_{max} = 0.5$, and $n$ is the Hill coefficient.

Results

The effects of buprenorphine on Na$^+$ currents were first examined on endogenous Na$^+$ channels in the DRG neuroblastoma hybridoma cell-line ND7/23. ND7/23 cells generate tetrodotoxin sensitive (TTXs) Na$^+$ currents and we recently demonstrated that the sensory neuronal $\alpha$-subunit Nav1.7 is expressed at high levels in these cells.21 As demonstrated in figure 1A, buprenorphine induced a concentration-dependent tonic block of Na$^+$ currents in ND7/23 cells. For resting Na$^+$ channels, which were examined in cells held at −140 mV, the IC$_{50}$ value for block by buprenorphine was 33 ± 2 μM (Hill coefficient 0.8 ± 0.07, n = 9). At the more physiologic holding potential −80 mV, however, the potency of buprenorphine for tonic block was significantly enhanced and gave an IC$_{50}$ value of 5 ± 0.5 μM (Hill coefficient 0.95 ± 0.07, n = 4) (P < 0.01, Student unpaired t test) (fig. 1B).

We next explored the effects of buprenorphine on the voltage-dependencies of activation and fast inactivation. As shown in figures 1C and D, application of 30 μM buprenorphine resulted in a strong reduction of the peak amplitude of Na$^+$ currents activated by depolarizing pulses ranging from −120 to 20 mV in steps of 10 mV. Buprenorphine induced a concentration-dependent hyperpolarizing shift of the voltage-dependency of activation ($V_{1/2}$ control: −22 ± 1.0 mV [n = 8], 3 μM buprenorphine: −27 ± 0.9 mV [n = 5], and 30 μM buprenorphine: −31 ± 0.8 mV [n = 8]) (fig. 1E). However, buprenorphine did not shift the reversal potentials (control: −20 ± 1 mV, 3 μM buprenorphine: −21 ± 2 mV, and 30 μM buprenorphine: 21 ± 1 μM) (P > 0.05 Student paired t test). We also observed a more prominent and concentration-dependent shift of the steady-state inactivation ($V_{1/2}$ control: −72 ± 0.3 mV [n = 6] by 3 μM buprenorphine: −79 ± 0.3 mV [n = 6] and 30 μM buprenorphine: −92 ± 0.7 mV [n = 6]) (fig. 1F). Buprenorphine also induced a pronounced use-dependent block of TTXs Na$^+$ channels in ND7/23 cells. In cells held at −140 mV, we observed a use-dependent reduction of Na$^+$ currents by 0.3 μM (14 ± 3%, n = 5), 3 μM (44 ± 5%, n = 6), and 30 μM (81 ± 4%, n = 5) buprenorphine when activated by 60 pulses at 10 Hz (figs. 2A and B). When tested with 3 μM buprenorphine, this use-dependent block became prominent at frequencies higher than 0.1 Hz (9 ± 2%) and marginally increased at rates of 0.5 Hz (21 ± 3%), 1 Hz (42 ± 2%), 2 Hz (44 ± 5%), and 10 Hz (46 ± 5%) (n = 5 or 6) (fig. 2C). At 30 Hz, a frequency which may occur in spontaneously active injured afferents, we observed a significant increased use-dependent even by 0.3 μM buprenorphine when compared with the effects at 10 Hz (63 ± 4% vs. 14 ± 3%) (fig. 2D) (P < 0.001, paired Student t test). Notably, activation at 30 Hz also resulted in a prominent Na$^+$ current reduction in control solution (11 ± 2%, n = 5) (fig. 2D).

The establishment of buprenorphine-induced block and the recovery upon washout was fairly slow. In order to quantify this observation, we monitored the effect of 300 μM buprenorphine applied for 100s on Na$^+$ currents activated at 0.1 Hz in cells held at −140 mV. As displayed in figure 3A, both the onset and the offset of buprenorphine-induced block lasted more than 1 min until a steady-state was established. Calculation of the time constants (τ) was performed by a fit with a single exponential function and revealed a τ$_{on}$ of 16 ± 1s and a τ$_{off}$ of 65 ± 1s. Notably, the LA lidocaine displayed considerable faster onset (τ$_{on}$ of 3 ± 1s) and offset (τ$_{off}$ of 5 ± 2s) (P < 0.01 for both onset and offset, unpaired Student t test) kinetics (fig. 3B). These data not only demonstrate that buprenorphine is a potent blocker of TTXs Na$^+$ channels, but also that buprenorphine-induced block of Na$^+$ channels is state-dependent and displays typical properties of LAs. We therefore asked if the effects of buprenor-
Phenylephrine on Na⁺ channels are because of a specific interaction with the LA-binding site. Use-dependent and tonic block by buprenorphine were examined on rat Nav1.4-wildtype and on the LA-insensitive mutants Nav1.4–1280°K, 434°K, and 1579°K.20,25,26 As is demonstrated in figures 4A–C, use-dependent block by 30 μM buprenorphine at 10 Hz during 60 pulse was more or less completely eliminated in the mutant Nav1.4–1579°K (15 ± 2%, n = 5) when compared with Nav1.4-wildtype (74 ± 3%, n = 6). Similar phenotypes were also observed for the Nav1.4–1280°K (21 ± 3%, n = 5) and Nav1.4–434°K (9 ± 4%, n = 5) (fig. 4C) (P < 0.001 for all three mutant constructs, unpaired Student t test.

**Fig. 1.** Tonic block of tetrodotoxin-sensitive Na⁺ currents in ND7/23 cells by buprenorphine. (A) Representative current traces of Na⁺ channels in presence of increasing concentrations of buprenorphine (V₉ = −140 mV). Currents were activated by 5 ms-long test pulses from −140 mV to 50 mV in intervals of 20 s. (B) Concentration-dependent block of resting Na⁺ channels examined at the holding potential (V₉ = −140 mV) and of inactivated channels examined at the physiologic V₉ = −80 mV. Peak amplitudes of Na⁺ currents at different drug concentrations were normalized with respect to the peak amplitude in control solution and plotted against the concentration of buprenorphine. The solid lines are fits of the data with the Hill equation. (C) Representative current traces of the voltage-dependent activation of Na⁺ channels in ND7/23 in control solution and (D) in presence of 30 μM. Cells were held at −140mV and currents were activated by depolarizing steps from −120 to 20 mV in steps of 10 mV. (E) Shift of the voltage-dependent activation of Na⁺ currents by buprenorphine at 3 and 30 μM. Currents were activated by 5 ms-long test pulses from −140 mV to −20 mV in steps of 10 mV. The conductance was calculated by the equation \( g_m = I_{Na}/(E_m - E_{rev}) \), where \( I_{Na} \) is the peak current, \( E_m \) is the amplitude of the voltage step, and \( E_{rev} \) is the reversal potential. The solid lines are fits by the Boltzmann function. (F) Shift of steady-state inactivation of Na⁺ currents by buprenorphine at 3 and 30 μM. Normalized currents of are plotted as a function of the conditioning prepulse potentials and the solid lines are fits of the data with the Boltzmann function. 100 ms-long prepulses from −140 to 10 mV in steps of 10 mV were followed by a 5-ms long test pulse to 50 mV. Cells were held at −140 mV. Bupr. = buprenorphine.
test). In contrast, tonic block of resting (V_h-140 mV) Nav1.4 channels was only marginally or not at all in the mutant constructs (IC_{50} wild-type 218 ± 60 µM, Hill 0.4 ± 0.1; 1579°K 237 ± 58 µM, Hill 0.5 ± 0.2; 1280°K: 98 ± 35 µM, Hill 0.6 ± 0.1, and 434°K 306 ± 46 µM, Hill 0.8 ± 0.1 (n = 5 or 6 for each construct) (fig. 4D).

Another hallmark of LA-induced state-dependent inhibition of Na^+ channels is a high affinity block of open chan-

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**Fig. 2.** Use-dependent block of tetrodotoxin-sensitive Na^+ currents in ND7/23 cells by buprenorphine. (A) Typical current trace of tetrodotoxin-sensitive Na^+ currents in ND7/23 activated by 10 Hz in presence of 30 µM buprenorphine. Cells were held at −140 mV and currents were activated by sixty 25 ms-long test pulses. Development of use-dependent block of Na^+ currents by 0.3, 3, and 30 µM buprenorphine at 10 Hz (B), by 3 µM buprenorphine at 0.1–10 Hz (C), and by control and 0.3 µM buprenorphine at 30 Hz (D). In B–D, peak currents were normalized to the first current and plotted against the pulse number. Bupr. = buprenorphine.

**Fig. 3.** Onset and offset of buprenorphine- (A) and lidocaine- (B) induced block of tetrodotoxin-sensitive Na^+ currents in ND7/23 cells. Cells were held at −140 mV and currents were activated by depolarizing pulses from −140 mV to 50 mV every 10 s. Peak amplitudes were normalized to the first test pulse and plotted against the time. Buprenorphine (300 µM) and lidocaine (1,000 µM) were applied for 100 s. Time constants for onset and offset of buprenorphine- and lidocaine-induced block were calculated by a fit with a single exponential function (dashed gray lines, values are given in the text). Bupr. = buprenorphine; Lido. = lidocaine.
channels, a feature that was recently suggested to be because of an inhibition of the voltage-sensor movement during activation. As wild-type $Na^+$ channels undergo fast inactivation within less than 1 ms, the inactivating-deficient mutant Nav1.4-WCW (L435W/L437C/A438W) was employed to explore the effects of buprenorphine on open $Na^+$ channels. As is demonstrated in figure 5A, Nav1.4-WCW $Na^+$ currents do not inactivate during a 50 ms-long test pulse from $-140$ to 50 mV. The blocking efficacy of buprenorphine on this persistent current (IC$_{50}$ value of $1.7 \pm 0.1 \mu M$, n = 8) was considerably higher than on the peak current measured at the beginning of the test-pulse ($19 \pm 5 \mu M$, n = 8) (fig. 5B) ($P < 0.01$, paired Student t test). As has been described previously in studies on Nav1.4-WCW with inhibitors like amitriptyline and flecainide, we also observed a time-dependent and concentration-dependent block during the 50 ms-long depolarization for which we sought to calculate the equilibrium dissociation constant (K$_D$). The decaying phase of the persistent Na$^+$ currents was fitted with a single exponential function and the resulting time constants were plotted against the concentrations of buprenorphine (fig. 5C). This plot was fitted with a linear regression, which revealed an on-rate (slope) of $10.5 \mu M^{-1} \cdot s^{-1}$ and off-rate (y-intercept) of $33.7 \mu M$. A K$_D$-value of 3.2 $\mu M$ was calculated by the equation K$_D$ = off-rate/on-rate.

Sensory neurons express several $Na^+$ channel $\alpha$-subunits, including Nav1.2, 1.3, 1.6, 1.7, 1.8, and 1.9. Although the LA-binding site is conserved in these $\alpha$-subunits, they can display significantly different sensitivities to block by LAs and other substances which interact with the LA-binding site. Thus we asked if buprenorphine induces a relevant subunit-specific inhibition of $Na^+$ channels (fig. 6). We found that buprenorphine induces a potent tonic (fig. 6A) and use-dependent (fig. 6C) block on human Nav1.7 expressed in Hek293t cells held at $-140$ mV. We found only small differences in tonic block between the neuronal subunits rat Nav1.3 (IC$_{50}$ 50 ± 5 $\mu M$, Hill 0.7 ± 0.1, n = 8), human Nav1.7 (IC$_{50}$ 47 ± 9 $\mu M$, Hill 0.5 ± 0.02, n = 9), and rat Nav1.8 (examined in presence of 300 nM TTX) (IC$_{50}$ 79 ± 15 $\mu M$, Hill 0.7 ± 0.1, n = 6), and the heart muscle subunit rat Nav1.5 (IC$_{50}$ 55 ± 9 $\mu M$, Hill 1.3 ± 0.1, n = 8) (fig. 6B). Thus a poor tonic block of rat Nav1.4 (218 ± 60 $\mu M$) seems to be the only subunit-specific effect of buprenorphine. Furthermore, all investigated subunits displayed a similar use-dependent block by 30 $\mu M$ buprenorphine at 10 Hz (fig. 6D).

We now asked if buprenorphine-induced inhibition of $Na^+$ channels can be demonstrated in more intact preparations. To address this question, the effects of buprenorphine...
on Na$^+$ currents in cultured DRG neurons and on the generation of action potentials in C-fibers were examined (fig. 7). Buprenorphine induced a concentration-dependent tonic block of Na$^+$ currents in DRG neurons held at −80 mV (IC$_{50}$ 22 ± 1 μM, n = 5) (figs. 7A and C). In order to examine the effect of buprenorphine on C-fibers, increasing concentrations of buprenorphine (more than 50 μM) were applied into the receptive field of single C-fibers which were electrically activated at 0.1 Hz. Buprenorphine blocked electrically evoked action potentials in 6 of 6 tested C-fibers and this effect reversed slowly upon washout (fig. 7B). Although three C-fibers were blocked by 300 μM, the other three were blocked by 100, 200, and 400 μM, respectively. Thus, although high concentrations were required to induce a total block of C-fibers, buprenorphine indeed displays local-anesthetic effects on intact nerves.

Although this is the first report on the inhibitory effect of buprenorphine on Na$^+$ channels, a number of previous reports have demonstrated an OR-independent inhibition of Na$^+$ channels by the opioids like meperidine, sufentanil, fentanyl, tramadol, and several synthetic κ-opioid receptor agonists. In order to compare the blocking potency of buprenorphine with that of other clinically relevant opioids, we explored tonic and use-dependent block of TTXs Na$^+$ channels in ND7/23 cells by buprenorphine, fentanyl, tramadol, morphine, and remifentanil (fig. 8). All five opioids inhibited TTXs Na$^+$ channels, however, with slightly different potencies (fig. 8). Sufentanil (IC$_{50}$ 111 ± 6 μM, Hill 1.1 ± 0.1, n = 6), fentanyl (IC$_{50}$ 95 ± 5 μM, Hill 1.0 ± 0.1, n = 8), tramadol (IC$_{50}$ 194 ± 9 μM, Hill 1.2 ± 0.1, n = 10), and morphine (IC$_{50}$ 378 ± 20 μM, Hill 1.0 ± 0.1, n = 8) blocked resting Na$^+$ channels in cells held at −140 mV in a concentration-dependent manner. Remifentanil induced an almost “all-or-none” blockade as only the highest concentration tested (1,000 μM) induced a robust block (IC$_{50}$ 612 ± 17 μM, Hill 2.4 ± 0.1 n = 10) (fig. 8I). Notably, buprenorphine exhibited a significantly more potent block as compared with all other examined opioids (P < 0.05 for all opioids, unpaired Student t tests). Use-dependent block by sufentanil (30 μM), fentanyl (30 μM), tramadol (100 μM), morphine (300 μM), and remifentanil (300 μM) were tested at 2 and 10 Hz (n = 5 or 6 cells for each substance). Morphine did not seem to induce a relevant use-dependent block (fig. 8H). Tramadol and remifentanil induced a moderate use-dependent block that did not increase with a faster activation rate from 2 to 10 Hz (fig. 8F and J). In contrast, both sufentanil and fentanyl induced a pronounced use-dependent block that increased with a faster activation rate from 2 to 10 Hz (fig. 8B and D).

**Discussion**

The present study identifies buprenorphine as a potent inhibitor of α-subunits of voltage-gated Na$^+$ channels. We present evidence that buprenorphine acts like a typical LA by targeting the “LA-binding site” of Na$^+$ channel α-subunits. This inhibition of Na$^+$ channels offers a plausible mechanism for the prolongation and enhancement of local anesthesia when buprenorphine is applied as an adjuvant to classic LAs. Furthermore, this property might be of relevance for the ability of buprenorphine to prevent development of hyperalgesia.
Voltage-gated Na\(^+\) channels are crucial for a proper electrogenesis in all excitable cells and they therefore constitute important targets for several substances employed in clinical practice, including LAs, antiarrythmics, antidepressants, and anticonvulsants. The determinants for the interaction of these substances with Na\(^+\) channels have been studied in detail and in most cases, their effects on Na\(^+\) channels seem to crucially depend on specific intracellular residues between the segment 5 and 6 in the domains one, three, and four of the pore-forming \(\alpha\)-subunits.\(^{20}\) The interaction with this "LA-binding site" depends on the conformational state of the \(\alpha\)-subunits, i.e., whereas a high-affinity inhibition occurs on inactivating and on open channels, the blocking efficacy is lower on channels residing in the closed state.\(^{20}\) Recent reports suggest that the high-affinity block of open and inactivated channels involves an inhibition of the voltage-sensor and should thus be termed "voltage-sensor block."\(^{27,28}\) Furthermore, the low-affinity closed-stated block was proposed to reflect an interaction of the blocker with the walls of the Na\(^+\) channel a neutral form of the drug interacting with neutral residues in the closed channel pore and could thus be termed "lipophilic block."\(^{28}\)

The present study is the first to demonstrate exactly these properties for buprenorphine. Tonic block of Na\(^+\) currents by buprenorphine was concentration-dependent and displayed a pronounced state-dependency with a high-affinity block of inactivated channels. A high affinity to inactivated channels first became evident when the holding potential was reduced from \(-140\) mV to \(-80\) mV. Importantly, \(-80\) mV is close to the physiologic resting membrane potential of sensory neurons and thus buprenorphine should induce a relevant tonic block already at high submicromolar concentrations \(\text{in vivo}\). Furthermore, a high affinity to inactivating channels was demonstrated by the experiments, which revealed that buprenorphine shifted the voltage-dependency of fast inactivation toward more hyperpolarized potentials in a concentration-dependent manner. A potent inhibition of open channels (i.e., a voltage-sensor block) by buprenor-

### Fig. 6. Block of neuronal and nonneuronal Nav\(\alpha\)-subunits by buprenorphine. (A) Representative current traces of Nav1.7 expressed in Hek293t cells in presence of increasing concentrations of buprenorphine (\(V_h = -140\) mV). Currents were activated by 5 ms-long test pulses from \(-140\) mV to 50 mV in intervals of 20 s. (B) Concentration-dependent block of Nav1.3, 1.4, 1.5, 1.7, and 1.8 by buprenorphine. Currents were activated by 5 ms-long test pulses from \(-140\) mV to 50 mV in intervals of 20 s. Peak amplitudes of Na\(^+\) currents at different drug concentrations were normalized with respect to the peak amplitude in control solution and plotted against the concentration of buprenorphine. Solid lines are fits of the data with the Hill equation. (C) Typical current trace of Nav1.7 activated by 10 Hz in presence of 30 \(\mu\)M buprenorphine. Cells were held at \(-140\) mV and currents were activated by sixty 25 ms-long test pulses. (D) Use-dependent block of Nav1.3, 1.4, 1.5, 1.7, and 1.8 by 30 \(\mu\)M buprenorphine. Currents were activated at 10 Hz with 25 ms-long pulses to 50 mV \(V_h = -140\) mV. Peak currents were normalized to the current of the first pulse and plotted against the pulse number. Bupr. = buprenorphine.

### Potent Block of Na\(^+\) Channels by Buprenorphine

Voltage-gated Na\(^+\) channels are crucial for a proper electrogenesis in all excitable cells and they therefore constitute important targets for several substances employed in clinical practice, including LAs, antiarrythmics, antidepressants, and anticonvulsants. The determinants for the interaction of these substances with Na\(^+\) channels have been studied in detail and in most cases, their effects on Na\(^+\) channels seem to crucially depend on specific intracellular residues between the segment 5 and 6 in the domains one, three, and four of the pore-forming \(\alpha\)-subunits.\(^{20}\) The interaction with this "LA-binding site" depends on the conformational state of the \(\alpha\)-subunits, i.e., whereas a high-affinity inhibition occurs on inactivating and on open channels, the blocking efficacy is lower on channels residing in the closed state.\(^{20}\) Recent reports suggest that the high-affinity block of open and inactivated channels involves an inhibition of the voltage-sensor and should thus be termed "voltage-sensor block."\(^{27,28}\) Furthermore, the low-affinity closed-stated block was proposed to reflect an interaction of the blocker with the walls of the Na\(^+\) channel a neutral form of the drug interacting with neutral residues in the closed channel pore and could thus be termed "lipophilic block."\(^{28}\)

The present study is the first to demonstrate exactly these properties for buprenorphine. Tonic block of Na\(^+\) currents by buprenorphine was concentration-dependent and displayed a pronounced state-dependency with a high-affinity block of inactivated channels. A high affinity to inactivated channels first became evident when the holding potential was reduced from \(-140\) mV to \(-80\) mV. Importantly, \(-80\) mV is close to the physiologic resting membrane potential of sensory neurons and thus buprenorphine should induce a relevant tonic block already at high submicromolar concentrations \(\text{in vivo}\). Furthermore, a high affinity to inactivating channels was demonstrated by the experiments, which revealed that buprenorphine shifted the voltage-dependency of fast inactivation toward more hyperpolarized potentials in a concentration-dependent manner. A potent inhibition of open channels (i.e., a voltage-sensor block) by buprenor-
The inhibitory effects of buprenorphine were only marginally different among these -subunits Nav1.3, Nav1.4, Nav1.5, Nav1.7, and Nav1.8. Consistent to the fact that the LA-binding site is well conserved in all Nav -subunits, the inhibitory effects of buprenorphine were only marginally different among these -subunits.

It should be noted that the potency of buprenorphine to block Na⁺ channels is considerably higher than that of lidocaine and even stronger than that of the most potent LA, bupivacaine, when examined under identical experimental conditions. These observations correlate well with the high lipophilicity of buprenorphine (octanol:water partition ratio approximately 2,000–100,000) and the fact that the lipophilicity is a major determinant for the blocking potency of LAs on Na⁺ channels. The same property is decisive for several pharmacokinetic properties, such as that buprenorphine dissociates very slow from ORs. The slow onset and offset of buprenorphine-induced tonic block of Na⁺ channels is probably because of the high lipophilicity as well, and this property should result in a local anesthetic block with slow on and off characteristics. Indeed, buprenorphine blocked the generation of action potentials in isolated C-fibers with a higher potency and with slower onset and offset kinetics as compared with lidocaine.

**Several Opioids Inhibit Voltage-gated Na⁺ Channels**

Although this is the first report on the inhibitory effect of buprenorphine on Na⁺ channels, previous reports have demonstrated an OR-independent inhibition of Na⁺ channels by several other opioids. The best studied opioid in terms of LA-like properties is probably meperidine, which was shown to block the skeletal muscle Na⁺ channel Nav1.4 in a state-dependent manner and via the LA-binding site. The blocking potency of meperidine on Nav1.4 was similar to that of lidocaine, thus the potency of meperidine is considerably lower than that of buprenorphine. Accordingly, the lipophilicity of meperidine (octanol:water coefficient approximately 39) is lower than that of buprenorphine. A more recent study from Haeseler et al. demonstrated that also sufentanil, fentanyl, and tramadol, but not morphine, block Nav1.2 in a state-dependent manner. Although not as potent as buprenorphine, we confirm here that sufentanil, fentanyl, and tramadol are indeed potent blockers of neuronal Na⁺ channels. In contrast to the reported failure of morphine to block Nav1.2, however, we observed a robust tonic block of TTX-sensitive Na⁺ channels in ND7/23 cells (i.e., Nav1.7). Our data are supported by previous studies on more intact preparations showing that morphine can block neuronal excitability in an OR-independent manner. It is thus possible that morphine displays a more pronounced subunit-specificity than that observed for buprenorphine in this study.

**Clinical Relevance for Buprenorphine and Other Opioids as Inhibitors of Na⁺ Channels**

Buprenorphine seems to be superior to other opioids for treatment of some types of chronic and neuropathic pain. If antihyperalgesia is decisive for these obviously favorable clinical features of buprenorphine, it should be of interest to explore the molecular targets mediating this effect. The main question behind this study was if inhibition of Na⁺ channels can explain the ability of buprenorphine to induce antihyperalgesia. Furthermore, we hoped to elucidate why most other opioids induce less or no antihyperalgesia although an inhibition of Na⁺ channels seems to be a com-
Fig. 8. Tonic and use-dependent block of tetrodotoxin-sensitive Na⁺ channels in ND7/23 cells by sufentanil (A, B), fentanyl (C, D), tramadol (E, F), morphine (G, H), and remifentanil (I, J). (A, C, E, G, I) For tonic block, currents were activated by 5 ms-long test pulses to 50 mV in intervals of 20 s from a holding potential of −140 mV. Peak amplitudes were normalized with respect to the peak amplitude in control and plotted against the drug concentration. Solid lines are fits of the data with the Hill equation. (B, D, F, H, J) For use-dependent block, currents were activated at 2 and 10 Hz with trains of 25 ms-long pulses to 50 mV in presence the respective drug. Peak currents were normalized to the current of the first pulse and plotted against the pulse number.
mon property of opioids.11 With the exception that remifentanil hardly blocked Na⁺ channels at all, our data suggest that the potencies of opioids to inhibit of Na⁺ channels (buprenorphine > fentanyl = sufentanil > tramadol > morphine > remifentanil) correlate well with the lipophilicity (buprenorphine > sufentanil > fentanyl > remifentanil > tramadol > morphine). In contrast, the blocking potency on Na⁺ channels and the analgesic potency did not correlate (sufentanil > fentanyl > remifentanil > buprenorphine > morphine > tramadol). It would of course be interesting to see if the potency of Na⁺ channel blockade correlates with the ability to induce antihyperalgesia. However, antihyperalgesia has not yet been investigated for sufentanil, fentanyl, and morphine in a human pain model. Considering our hypothesis, we would predict that sufentanil and fentanyl, but not morphine, will have significant antihyperalgesic properties.

For tramadol, Filitz et al. demonstrated a negligible antihyperalgesic effect in a human pain model.42 Remifentanil, on the other hand, induces no antihyperalgesia, but rather an acute postinfusion hyperalgesia.43 Thus, with a strongly limited number of investigated opioids, we indeed see a correlation between the ability to block Na⁺ channels and to induce antihyperalgesia.

In patients with high-dose buprenorphine patches, maximal plasma concentrations of 700 pg/ml (approximately 2 nM) have been described.13 Taking our in vitro data into account, it can be doubted that a relevant block of Na⁺ channels occurs at clinically relevant systemic concentrations. Importantly, though, the plasma level of an extremely lipophilic substance like buprenorphine does not necessarily reflect the effective concentration in the peripheral and central nervous system. Furthermore, a robust antihyperalgesia was induced by the less lipophilic LA lidocaine at maximal plasma levels of approximately 15 µM.44,45 In this range, lidocaine induces a small but significant block of inactivated and open Na⁺ channels.22,46 Furthermore, lidocaine is known as a potent use-dependent blocker and this property could as well prevent the development of hyperalgesia.22 The ability of buprenorphine to directly inhibit neuronal excitability or to enhance and prolong the effect of classic LAs has been reported on in several previous reports.17,18 In clinical practice, buprenorphine is indeed one of few effective adjuvants when applied together with LAs for a peripheral nerve block.47 It seems likely that the direct inhibition of Na⁺ channels is an important, if not essential, mechanism for these properties.

In summary, we show for the first time that buprenorphine is a potent inhibitor of voltage-gated Na⁺ channels and can be classified as a strong LA. Buprenorphine shares this property with several other opioids but is by far the most potent blocker of Na⁺ channels. This LA action adds to an already complex pharmacological diversity of buprenorphine and offers another mechanism by which buprenorphine can induce analgesia and antihyperalgesia.

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