Sodium channels (from rat, mouse, and man) in neuroblastoma cells and different expression systems have similar sensitivities to pentobarbital

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Abstract

Current understanding of the molecular mechanisms of anaesthesia entails multiple molecular sites of anaesthetic action, each more or less important for a specific anaesthetic drug used. For some molecular anaesthetic targets such as the GABA_A [4,2], and acetylcholine receptors [5] as well as potassium channels [3], large differences in anaesthetic effects and sensitivity between different receptor subtypes have been found. Voltage-gated sodium channels have been shown to be affected at clinical concentrations of pentobarbital [12]. However, these results were obtained in an expression system, and anaesthetic sensitivity may be dependent on sodium channel subtype and subunit composition, too. Therefore, we compared the sensitivity of voltage-gated sodium channels to pentobarbital in human (SH-SYSY) and mouse (N1E-115) neuroblastoma cell lines as well as an HEK293-cell expression system with previously reported data with other sodium channel subtypes. Remarkably, for all sodium channel subtypes studied as well as all subtypes reported in the literature, pentobarbital had qualitatively identical effects with some quantitative differences, and at potentials near the action potential threshold sodium currents were significantly reduced by clinical concentrations of pentobarbital.

Keywords: General anaesthesia patch-clamp; Pentobarbital; Sodium channel neuroblastoma; Expression system

The molecular mechanisms of anaesthetic action are still not well understood. General anaesthetics, including barbiturates, exert effects on a wide variety of receptors and ion channels [8]. Current understanding favours the modulation of GABA_A-receptors as the primary mechanism of action of barbiturates, by direct activation of the receptor at clinical drug concentrations or potentiating GABA evoked responses at even lower concentrations. However, it has been shown that binding affinities for barbiturates are dependent on subunit composition of the GABA_A-receptors [2], and that some subtypes are even insensitive to anaesthetics [4]. Similar differences have been reported for acetylcholine receptors [5] as well as potassium channels [3]. Recently, it has been shown that voltage-gated sodium channels can be blocked by clinical concentrations of pentobarbital [12] as well as volatile anaesthetics [14]. The findings were obtained with the rat brain IIa sodium channel subtype expressed in a Chinese hamster ovary (CHO)-cell expression system. Like potassium channels [3], GABA_A [2], and acetylcholine receptors [5], different sodium channel subtypes may exhibit different anaesthetic sensitivity, and anaesthetic effect may be modified by the lack of the β-subunits in the expression system. Since it is presently not possible to perform intracellular recordings in vivo, we tested the effect of pentobarbital on sodium currents in a human (SH-SYSY) and a mouse (N1E-115) neuroblastoma cell lines. In addition, we studied a stably transfected human embryonic kidney (HEK293) cell line expressing human muscle type (hSkM1) sodium channels, and compared the results with the published data from...
the CHO-cell expression system and from other experimen-
tal systems. This selection thus includes channel subtypes
from different species, different tissues, and different
experimental systems (neuroblastoma cells versus expres-
sion system).

NIE-115 cells were grown in DMEM medium (Gibco)
containing 10% foetal bovine serum (Biochrom KG) and
fetal bovine serum (Biochrom KG) in RPMI medium (Gibco)
containing 10% foetal bovine serum (Biochrom KG) and 130
mmol/L CsCl, 10 mM NaCl and 5 mM HEPES, adjusted to pH
7.4 with CsOH. All solutions were filtered through 0.45
mmol/L (pore size) syringe filters.

The recordings were made at room temperature (21 ±
°C). Sodium currents were studied using the whole-cell
configuration of the patch-clamp recording technique [9].
Currents were recorded by a standard patch-clamp amplifier
(LPC7, List, Darmstadt, Germany), controlled by commer-
cially available software (pClamp; Axon Instruments) on a
standard PC. Currents were filtered at 10 kHz. Capacitative
transients and series resistance were measured and
compensated using the internal compensation cir-
cuitry of the amplifier, when possible.

Mean cell capacities were 42.9 ± 17.2, 43.6 ± 16.9, and
7.9 ± 3.0 nA (all data are reported ±SD). Series resistance compensation of
up to 60% was used.

Patch-clamp pipettes for all experiments were filled with
an intracellular solution containing 90 mM CsF, 60 mM
CsCl, 10 mM NaCl and 5 mM HEPES, adjusted to pH 7.4 with
CsOH.

Pentobarbital (sodium salt, Sigma) was dissolved directly
in the extracellular solution and applied via perfusion pip-
ette positioned close to the cell. Solutions with pentobarbital
greater than 3.4 mM were readjusted to a pH of 7.4 with
HCl.

A cell line exhibited a similar voltage dependence of
sodium current activation and inactivation (Fig. 1A).

Pentobarbital effects were characterised as described pre-
viously [12]. Peak current suppression was measured as
reduction of the maximum inward sodium current obtained
from standard current-voltage plots. Currents were elicted
by stepping the potential from a holding value of -100 mV
(CHO-cells, SH-SYSY cells) or -85 mV (HEK- and NIE-
15 cells) to test potentials ranging from -60 to +80 mV. Pen-
tobarbital effect was reversible in all experiments, and the
average of prepentobarbital and washout values was used as
control value.

Data from 3–7 experiments for each pentobarbital con-
centration (total of 31 for NIE-115, 11 for SH-SYSY, and
21 for HEK293) were used to establish concentration-
response curves for pentobarbital suppression of maximum inward currents. Lines are fits of a Hill function to the data. Data points are
averages of 3-5 experiments for SH-SYSY and HEK293 cells, and 5-6 experiments for NIE-115 cells.
Comparison of the parameters of the concentration-response curves of pentobarbital for maximum inward current suppression in whole-cell patch-clamp experiments and concentration-response data from other experimental systems (mean ± SE of the fit).

Table 1

<table>
<thead>
<tr>
<th>Ref.</th>
<th>EC50 ± SE</th>
<th>Hill coefficient ± SE</th>
</tr>
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<tbody>
<tr>
<td>White-cell clamp</td>
<td></td>
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<tr>
<td>SH-SYSY</td>
<td>3.04 ± 0.38</td>
<td>0.85 ± 0.17</td>
</tr>
<tr>
<td>NIE-115</td>
<td>2.54 ± 0.43</td>
<td>1.10 ± 0.20</td>
</tr>
<tr>
<td>HEK293</td>
<td>1.95 ± 0.50</td>
<td>1.87 ± 0.53</td>
</tr>
<tr>
<td>CHO brain</td>
<td>1.49 ± 0.06</td>
<td>0.95 ± 0.05</td>
</tr>
<tr>
<td>CHO muscle</td>
<td>1.18 ± 0.11</td>
<td>0.64 ± 0.10</td>
</tr>
<tr>
<td>Lipid bilayer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human brain [7,13]</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Human heart [9]</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Human skeletal [16]</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eel electroplax [17]</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Other preparations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squid axon [6]</td>
<td>-1.0</td>
<td></td>
</tr>
<tr>
<td>Frog muscle [15]</td>
<td>0.9</td>
<td></td>
</tr>
</tbody>
</table>

Response curves (Fig. 2B) as fits of a Hill function: $EC_{50} = \frac{100 - (100*P/P_{max})}{1 + e^{(P/P_{max})}}$, where $P$ is the prepulse potential, $P_{max}$ is the prepulse potential at which $P$ is at a maximum, and $EC_{50}$ is the concentration of pentobarbital required to reduce the maximum inward current to 50% of control.

The concentration-response curves of SH-SYSY-cells and NIE-115-cells were not significantly different, neither were those of the CHO-cells expressing brain and muscle channels. However, a significant difference was found between SH-SYSY-cells and NIE-115-cells on one side and the two CHO-cell lines on the other. The sodium channels in the two neuroblastoma cell lines are significantly less sensitive to pentobarbital than the sodium currents in any of the other cell lines.

In addition to the reduction of maximum inward current, we characterized the effect of pentobarbital on sodium channel steady-state availability. Steady-state availability was assessed by a two-pulse protocol comprising a 500 ms prepulse (long enough to allow channel availability to reach a steady state) to potentials ranging from -150 to -10 mV, followed by a test pulse to -10 mV.

Channel availability plots reveal two effects of pentobarbital: (1) a voltage-independent current suppression at hyperpolarized potentials and (2) a shift of steady-state inactivation in the hyperpolarizing direction (Fig. 2A). Both effects combine to lead to a voltage-dependent current inhibition by pentobarbital, as described previously for sodium channels in the CHO-cell lines. Averaged channel availability plots from all experiments were used to establish concentration-response curves at each prepulse potential.

The EC50 values of these curves are shown in Fig. 2B, demonstrating the voltage dependence of pentobarbital potency. At hyperpolarized potentials, EC50 values range from 1.2 to 2.38 mM.
to 2.4 mM and are similar to the values reported for planar lipid bilayers under conditions where inactivation is removed (Table 1): 0.1 mM for human brain (synaptosomes) [7,13] and for human cardiac-type sodium channels [16], about 1.0 mM for human skeletal muscle [16], and about 0.6 mM for the muscle-type sodium channels from red blood cell ghosts [17]. Similar values have also been reported for sodium channels of squid axon [6] and frog muscle [15].

At -60 mV, a potential between resting potential and neuronal firing threshold, maximum inward sodium current block by concentrations reached during clinical use of pentobarbital (200 μM) [1] ranges from 12.5% in SH-SYSY neuroblastoma cells to 40.0% in CHO-cells. Additional time-dependent block [12] may lead to even larger functional blockade.

At equal membrane potentials, differences in potency between the sodium channel subtypes may be due to the link of beta-subunits in the expression systems [10], differences in sodium channel structure, or differences in the cellular environment of the channels. In addition, post-translational modification of sodium channels of ten different subtypes considered here may vary by about 0.6 mM for the muscle-type sodium channels from red blood cell ghosts [17] to 2.4 mM and are similar to the values reported for planar lipid bilayers under conditions where inactivation is removed (Table 1): 0.1 mM for human brain (synaptosomes) [7,13] and for human cardiac-type sodium channels [16], about 1.0 mM for human skeletal muscle [16], and about 0.6 mM for the muscle-type sodium channels from red blood cell ghosts [17]. Similar values have also been reported for sodium channels of squid axon [6] and frog muscle [15].

At -60 mV, a potential between resting potential and neuronal firing threshold, maximum inward sodium current block by concentrations reached during clinical use of pentobarbital (200 μM) [1] ranges from 12.5% in SH-SYSY neuroblastoma cells to 40.0% in CHO-cells. Additional time-dependent block [12] may lead to even larger functional blockade.

At equal membrane potentials, differences in potency between the sodium channel subtypes may be due to the link of beta-subunits in the expression systems [10], differences in sodium channel structure, or differences in the cellular environment of the channels. In addition, post-translational modification of the channels may be different in neuroblastoma cells and the cell lines used in the expression systems. Despite these quantitative differences, sodium channels of ten different subtypes considered here vary by about 0.6 mM for the muscle-type sodium channels from red blood cell ghosts [17] to 2.4 mM and are similar to the values reported for planar lipid bilayers under conditions where inactivation is removed (Table 1): 0.1 mM for human brain (synaptosomes) [7,13] and for human cardiac-type sodium channels [16], about 1.0 mM for human skeletal muscle [16], and about 0.6 mM for the muscle-type sodium channels from red blood cell ghosts [17]. Similar values have also been reported for sodium channels of squid axon [6] and frog muscle [15].