SH-SY5Y Cells: A Model for the Study of Pharmacological Action on Human Nicotinic Acetylcholine Receptors of Sympathetic Ganglions

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Introduction

General anesthetic agents may cause severe decreases in blood pressure during the induction of general anesthesia. This has been suggested to result from direct dilatory effects on blood vessels, from direct negative inotropic effects on the heart, as well as from inhibition of the sympathetic nervous system. Acetylcholine is the main transmitter in ganglionic cells of the peripheral nervous system. By stimulating postsynaptic nicotinic acetylcholine receptors acetylcholine chemically transmits electrical sympathetic activity. Inhibition of these ion channels would consequently depress sympathetic nerve discharge and may, thus, contribute to decreases in blood pressure observed during general anesthesia.

The human neuroblastoma cell line SH-SY5Y resembles sympathetic neurons in culture. It is genetically stable, shows positive for neuron-specific enolase, and shows activity of both dopamine-ß-hydroxylase and choline-acetyltransferase. It contains vesicles storing epinephrine andnorepinephrine, and norepinephrine is released by nicotinic receptor stimulation. As established by PCR, SH-SY5Y cells express mRNA for α3, α5, α7, β2, and β4 subunits. Northern blot experiments demonstrate mRNA for the α3, α7, β2, and β4 subunits with intensities of the hybridisation signal α3 > β3 > β2 > α7. According to Wang et al., most of the functional receptors in SH-SY5Y cells consist of an α3β2 or an α3β4 combination, thus resembling the expression pattern of...
nicotinic acetylcholine receptors in sympathetic ganglion cells. SH-SY5Y cells, therefore, constitute a valid model for the study of anesthetic action on ganglionic cells of the sympathetic nervous system.

The aim of this study was to investigate if nicotinic acetylcholine receptors natively expressed in human neuroblastoma SH-SY5Y cells allow the investigation of anesthetic action on these ion channels. The results of this ongoing study may help to elucidate why general anesthetic agents cause severe changes in blood pressure during the induction of general anesthesia.

Methods/Results

SH-SY5Y cells were grown using RPMI medium (Biochrom, Berlin, Germany) at 37°C with 95% air and 5% CO₂. Growth medium contained 10% fetal calf serum (Biochrom, Berlin, Germany), penicillin (100 U/ml) and streptomycin (100 μg/ml) (Life Technologies, Paisley, Scotland). Neuronal differentiation was induced by exposure to 10 μM retinoic acid (Sigma, Deisenhofen, Germany) for 3-7 days.

Whole cell patch clamp recordings were performed with an EPC7 amplifier (List, Darmstadt, Germany). Nicotinic Ach-receptor currents were evoked by applying 1 mM Ach for 1 s at a holding potential of -60 mV. The concentration of Ach (Sigma, Deisenhofen, Germany) was chosen because it results in a maximal current response of nAChRs. Extracellular solution consisted of (in mM): NaCl, 150; KCl, 5.6; CaCl₂ 1.8; MgCl₂ 1; HEPES, 10; pH, 7.4. Recording pipettes with resistances of 2.5-4 MΩ were filled with intracellular solution (in mM): KCl, 140; EGTA, 10; MgCl₂, 3; HEPES, 10; pH, 7.4. All constituents of the intra- and extracellular fluids were purchased from Sigma (Deisenhofen, Germany). Patch pipettes were prepared from Borosilicate glass capillaries (Kwik-Pip, WPI, USA) with a two stage pipette puller and a piezoforge (LM-3P-A and LIM CPZ-101, both List, Darmstadt, Germany). Experiments were performed at room temperature. The effects of anesthetic agents on the nAChR currents were studied in a continuous exposure of the measured cells the drug before and during application of Ach (1 mM). Current analysis was performed with pClamp software (Version 6.0.4, Clampfit, Axon Instruments, Foster City, CA, USA). Inhibition was measured as peak current inhibition of nAChRs.

SH-SY5Y cells grow in a non-confluent monolayer. They can, therefore, be studied with the patch-clamp technique without electrical coupling between individual cells. The nAChR-mediated currents in these cells show a concentration-response curve characterized by a Hill coefficient of 1.9 and an EC₅₀-value for Ach of 100 μM (figure 1). Ach at 1 mM caused maximal current activation and induced peak whole-cell currents of 144 ± 16 pA (n = 25 cells). The currents were insensitive to α-bungarotoxin.

None of the anesthetics (+ ketamine, ketamine, propofol, isoflurane, thiopental, pentobarbital, etomidate) induced currents in SH-SY5Y cells (figure 2). The anesthetic agents suppressed Ach induced currents in a concentration-dependent and reversible manner (figure 3). The concentration-dependent inhibitory effects of the anesthetics on peak current inhibition of nAChRs.
Fig. 1: The nAChRs in SH-SY5Y cells show an agonist concentration-response curve characterized by a Hill coefficient of 1.9 and an EC50-value for ACCh of 100 μM. ACCh at 3 mM caused maximal current activation and induced peak whole-cell currents of an average 140 pA.

nAChR currents were fitted by Hill functions. The approximate half-maximal concentration for inhibition of nACh induced peak current are given in table 1. The rank order of potency at the ACCh were: ketamine > ketamine > ketamine > propofol > thiopental > pentobarbital > iopentane. The effects of ketamine were stereospecific.

Discussion

Northern blot experiments as well as experiments with the polymerase chain reaction demonstrate that most of the functional nAChRs in the SH-SY5Y cell line consist of an α3β2 and α3β4 subunit combination. The same nAChR-subunits can be identified in sympathetic ganglion cells of rat, chicken, and in the rat PC12 cell line. Human SH-SY5Y cells, like e.g. chick ciliary ganglion neurons, contain mRNA of α7 subunits, but their level of expression is low. The nAChR-subunits in SH-SY5Y cells, like in chick ciliary ganglion, assemble to ACChRs which do not bind α-bungarotoxin. Despite more abundant expression of α3β2 and α3β4 nAChR-subunits in the peripheral nervous system these subunits are not limited to the periphery and pharmacologic effects may be attributed to interaction with these subunits present in central neurons, as well. Nonetheless, SH-SY5Y cells constitute a valid model for a peripheral sympathetic neuron.
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A. Acetylcholine or thiopental

B. Acetylcholine or pentobarbital

C. Acetylcholine or isoflurane

D. Acetylcholine or propofol

E. Acetylcholine or ketamine

F. Acetylcholine or ethanol

Fig. 2: Thiopental (100 μM), pentobarbital (300 μM), propofol (400 μM), isoflurane (2.8 mM), ketamine (100 μM) and ethanol (1.7 mM) did not induce currents in SH-SY5Y cells. The arrow indicates application of the respective anesthetic. Control currents are activated by 1mM Ach.
Fig. 3: All anesthetics inhibited the nAChRs in SH-SYSY cells. Exemplary current traces show the effects of R(-) ketamine and S(+)-ketamine.

Tab. 1: Approximate IC₅₀-values derived from the concentration response data of peak nACh-receptor current inhibition by the anesthetic agents.

<table>
<thead>
<tr>
<th>Anaesthetic</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentobarbital</td>
<td>60</td>
</tr>
<tr>
<td>Thiopental</td>
<td>25</td>
</tr>
<tr>
<td>Propofol</td>
<td>15</td>
</tr>
<tr>
<td>Ketamine</td>
<td>1.5</td>
</tr>
<tr>
<td>Ketamine + Ketamine</td>
<td>1</td>
</tr>
<tr>
<td>Ketamine - Ketamine</td>
<td>1.5</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>700</td>
</tr>
</tbody>
</table>

The IC₅₀-value for nAChR inhibition in SH-SYSY cells by intravenous anesthetics reflect different affinities of these nAChRs than those in rat PC12 cells[10,21]. This difference in anesthetic sensitivity may be explained by the use of different agonists in both cell lines, nicotine versus ACh, by different subunit compositions in both cell lines, or and perhaps more importantly by species differences. Human neuronal nAChR β3 and β4 subunits, for example, only show 94% and 89% amino acid sequence identity compared to the rat neuronal nAChR β3 and β4 subunits, respectively[10,21].

Conclusion

Differences in the regulation of sympathetic activity between different species[10,21], as well as structural differences between nAChRs from different species[10,21] make it unclear if animal data can be extrapolated to human beings. As far as nAChRs are con-
censed the investigation of human cellular models, therefore, seems warranted. Human neuronal SH-SY5Y cells are easy to culture by standard protocols, and the α7nAChRs are amenable with the patch clamp technique. These cells, thus, constitute a suitable model to evaluate anesthetic effects on ligand-gated nACh-receptors natively expressed in human sympathetic ganglion like cells.

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