Role of α5-containing GABA(A) Receptors in Mediating Benzodiazepine Actions in Neocortical Circuits

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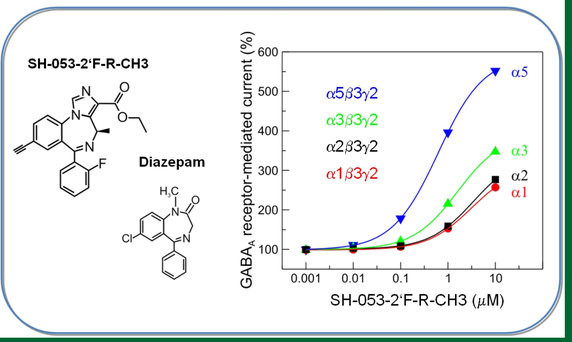
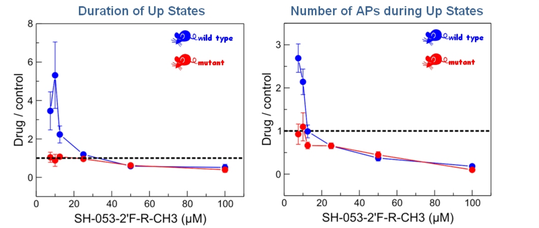
Introduction

BENZODIAZEPINES in current clinical use modulate a multitude of GABA(A) receptor-subtypes in the brain. Therefore, more specific benzodiazepine site ligands are expected to show an improved side effect profile.
The selectivity and efficacy of a novel compound can be evaluated in oocytes, expressing defined types of GABA(A) receptors. Further insights into the actions of the drug in the brain is provided by genetically modified mice, in which the putative drug target, a specific subtype of the GABA(A) receptor, has been rendered insensitive to the drug by a point mutation.
The newly synthesized benzodiazepine-site-ligand SH-053-2'F-R-CH$_3$ is most effective at GABA(A) receptors containing α5 subunits. These receptors are assumed to play a key role in memory formation and anesthetic-induced amnesia. Here we ask how SH-053-2'F-R-CH$_3$ affects the activity of neocortical neurons in brain slices from wild type and α5(H105R) knock in mice.

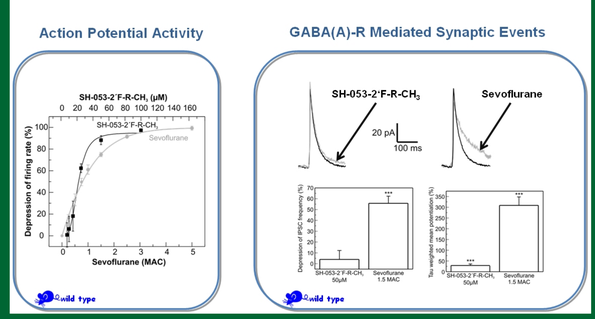
Structural Formula and Modulation of Different GABA(A) Receptor Subtypes



Effects of SH-053-2'F-R-CH$_3$ in Slices from Wild Type and α5(H105R) Knock In Mice



Different Actions of SH-053-2'F-R-CH$_3$ and Sevoflurane on GABA(A) Receptor-mediated Currents



Results: Properties of SH-053-2'F-R-CH$_3$

Efficacy

Oocytes	Neocortical Brain Slices
2-5 fold enhancement of GABA(A) receptor-mediated currents at 1-10 μM.	At 7.5-12 μM: Significant changes in neuronal activity patterns. Higher concentrations: Strong depression of action potential activity.

Selectivity

Oocytes	Neocortical Brain Slices
α5 > α3=α2=α1	Up to 12 μM: effects are abolished by the α5(H105R) mutation. Higher concentrations: Drug selectivity is lost.

Left: Structural formula of SH-053-2'F-R-CH$_3$ and diazepam.

Right: Concentration-effect curves for SH-053-2'F-R-CH$_3$ on different GABA(A) receptor subtypes, using an EC$_x$ GABA concentration. Data points represent means ± SEM.

Methods: cRNAs encoding GABA(A) receptor subunits were injected in stage 5-6 oocytes from Xenopus laevis and incubated for at least 36 h. SH-053-2'F-R-CH$_3$ was preapplied before GABA was added, which was coapplied until a peak response was observed.

A representative extracellular recording carried out in neocortical slices from wild type mice. SH-053-2'F-R-CH$_3$ prolonged episodes of ongoing activity (up states) but also periods of neuronal silence.

Methods: Tissue slices were prepared from 2-4 days old mice and cultured for 2-3 weeks. Voltage recordings were highpass filtered at 40 Hz in order to detect single- and multi-unit activity.

Left: Comparison on the effects of SH-053-2'F-R-CH$_3$ on the duration of cortical up states in different genotypes. In wild type, but not mutant mice concentrations up to 12.5 μM significantly prolonged this parameter (t-test, $p<0.05$, $n>8$). At concentrations >= 25 μM, SH-053-2'F-R-CH$_3$ decreased up state duration. In this concentration range, no difference was observed between wild types and mutants, indicating loss of drug selectivity. Data points represent means ± SEM.

Right: Effects on the average number of action potentials per up state were statistically significant at 7.5 and 10 μM.

Actions of SH-053-2'F-R-CH$_3$ on action potential activity during up states. The upper curves represent grand averages, in the presence and absence of the drug. In slices from wild type mice, SH-053-2'F-R-CH$_3$ enhances neuronal firing. This effect slowly develops in time and is absent in mutant mice (lower panels).
Green dots: In these bins, action potential firing in the presence of SH-053-2'F-R-CH$_3$ is different from control conditions. This effect is highly significant ($p<0.001$). Yellow dots: For these bins p is in between 0.05 and 0.001.

50 μM SH-053-2'F-R-CH$_3$ and 1.5 MAC sevoflurane attenuate action potential activity to a very similar degree (left panel, arrow). However, at these concentrations, both agents alter GABA(A) receptor-mediated inhibition in different ways. Sevoflurane produces a prominent prolongation of synaptic currents which is almost absent with the SH-compound. Furthermore, sevoflurane but not SH-053-2'F-R-CH$_3$ decreases the frequency of spontaneous IPSCs. These findings indicate that at 50 μM, SH-053-2'F-R-CH$_3$ acts mostly via non-α5-containing extrasynaptic receptors, which are expressed on pyramidal neurons, but not GABAergic interneurons.

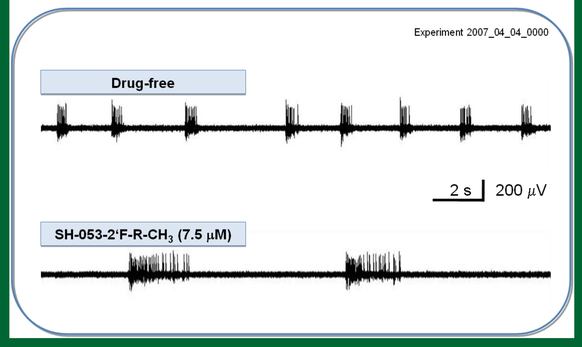
Key findings to be explained:
1) Positive modulation of α5-containing receptors by SH-053-2'F-R-CH$_3$ (10 μM) increases neuronal activity during up states.
2) GABAergic neurons lack α5-subunits.

Explanation:
α5-subunits are expressed in a sub-population of pyramidal cells. These cells have a strong impact on the activity of GABA-releasing interneurons. If these pyramidal cells are inhibited by the SH-compound, the activity of GABA-releasing neurons is also decreasing, causing disinhi-bition of a large population of excitatory pyramidal cells.

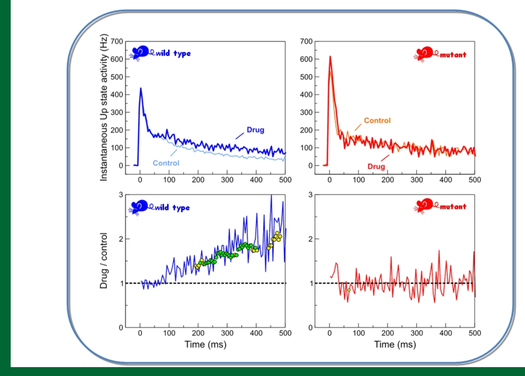
Results: Actions on Neocortical Neurons

1	Selective potentiation of α5-containing GABA(A) receptors *enhances* action potential firing of neurons in neocortical networks during up states and prolongs up state duration.
2	Subtype-unspecific upregulation of GABA(A) receptor function (at 25-100 μM SH-053-2'F-R-CH$_3$) decreases neuronal activity in a concentration-dependent manner.
3	SH-053-2'F-R-CH$_3$ does not change the amplitude and frequency of GABA(A) receptor-mediated events (even at very high concentrations), suggesting that GABAergic interneurons do not express α5-subunits.
4	At equipotent concentrations of sevoflurane and SH-053-2'F-R-CH$_3$, causing a similar depression of action potential activity, the latter agent is much less effective in prolonging GABA(A)-receptor-mediated synaptic currents, indicating that this compound acts mostly via extrasynaptic receptors.

Spontaneous Action Potential Activity in Neocortical Slices from Wild Type Mice



SH-053-2'F-R-CH$_3$ (10 μM) Enhances Action Potential Activity During Up States



Discussion