Application Note

Channel:	hGABA$_A$ α5β3γ2
Cells:	HEK293
Tools:	Port-a-Patch

Activation and inhibition of GABA$_A$ α5β3γ2 receptors on the Port-a-Patch

The electrophysiology team at Nanion Technologies GmbH, Munich.
Cells were kindly supplied by Merck Millipore.

Summary

Gamma aminobutyric acid type A (GABA$_A$) receptors are the most important inhibitory neurotransmitter receptors in the mammalian central nervous system (CNS). They are opened by GABA allowing the passage of chloride ions across the membrane. GABA$_A$ channels are modulated by a variety of different drugs including benzodiazepines, barbiturates, neuroactive steroids, anesthetics, and convulsants[1]. The receptors are heteropentameric and depending on the subunit combination, they exhibit different electrophysiological and pharmacological properties[2]. Six α-, three β-, three γ-, one δ-, one ε-, one π-, one θ- and three ρ-subunits have been cloned, including splice variants of some of these subunits[3,4]. Functional GABA$_A$ receptors typically assemble with two α, two β, and one γ subunit, with alternating α and β subunits connected by a γ subunit[1]. GABA$_A$ receptors play a critical role in regulating excitability of the brain, anxiety, vigilance, as well as learning and memory[2]. The α5 subunit is highly expressed in the hippocampus and olfactory bulb and expressed in low levels in other brain regions including the cortex, subiculum, hypothalamus, sympathetic preganglionic neurons, and amygdala[5]. GABA$_A$ receptors containing the α5 subunit cluster at both extrasynaptic sites as well as synaptic sites thus contributing to tonic currents and synaptic GABA-ergic neurotransmission[5]. α5 -containing receptors exhibit unique physiology and pharmacology and they are potential pharmacological targets for the treatment of neurodevelopmental disorders, depression, schizophrenia, and mild cognitive impairment.

The Port-a-Patch with External Perfusion System was used to record α5β3γ2 receptors expressed in HEK293 cells.

Results

GABA$_A$ receptors with the subunit combination α5β3γ2 were activated by increasing concentrations of GABA (Figure 1). Currents started to activate at concentrations above 3 µM and reached maximum at a concentration of 30 µM. A concentration response curve was constructed for an average of 8 cells (Figure 1B). The EC$_{50}$ was calculated to be 10.8 ± 0.2 µM (n = 8), in good agreement with the literature for cloned GABA$_A$ receptors expressing the α5-subunit[6].

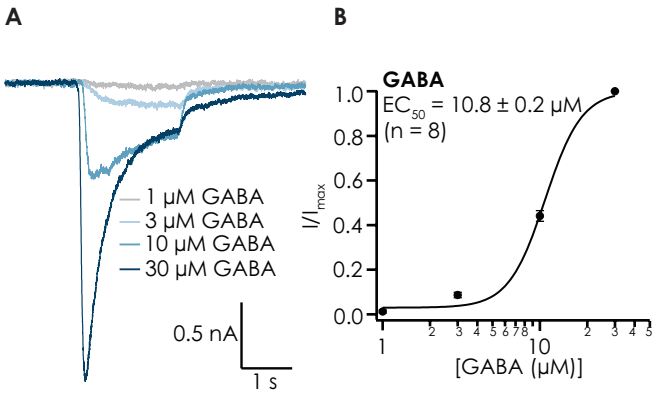

Figure 1: A Whole cell current responses from HEK293 cells expressing α5β3γ2 by increasing concentrations of GABA. Shown are current responses from an example cell with perfusion of external GABA at the concentrations indicated for 2 s. Following this, GABA was washed away by external recording solution. Holding potential was -80 mV. **B** Concentration response curve for an average of 8 cells was constructed and is shown. EC$_{50}$ = 10.8 ± 0.2 µM (n = 8).

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$GABA_A$ currents mediated by α5β3γ2 could be repetitively activated by 1 mM GABA (Figure 2). A small decrease in peak amplitude was observed between the first and second application of GABA (14% reduction from -525 ± 91 pA to -455 ± 65 pA). Following this, repeated application of GABA resulted in identical peak current amplitudes (< 3% difference compared with the previous amplitude).

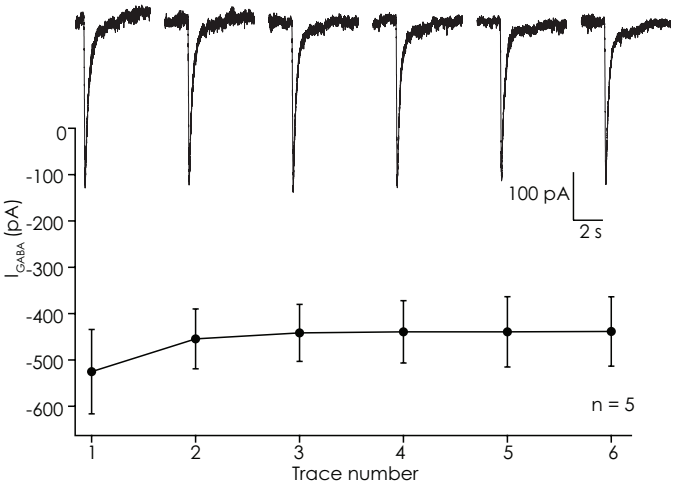

Figure 2: Traces from an example cell showing repetitive activation by 1 mM GABA (top) and the corresponding peak amplitudes for an average of 5 cells (bottom).

$GABA_A$ receptors with the subunit combination α5β3γ2 were activated by 30 µM GABA and blocked by the competitive antagonist, bicuculline, when co-applied with GABA (Figure 3).

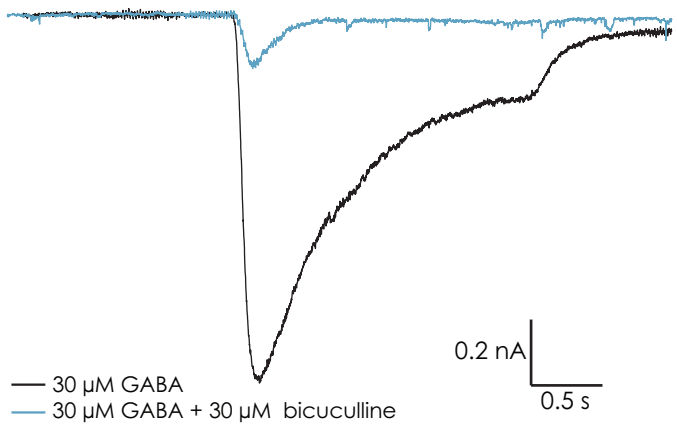

Figure 3: α5β3γ2 $GABA_A$ receptors activated by 30 µM GABA were blocked by 30 µM bicuculline when co-applied.

In conclusion, $GABA_A$ receptors with the subunit combination α5β3γ2 were reliably recorded on the Port-a-Patch with External Perfusion System. Perfusion of GABA was triggered automatically by the amplifier and repetitive activation of $GABA_A$ receptors was possible using a high concentration of GABA. In this way, both inhibitors, such as bicuculline, and other modulators (not shown) could be co-applied and characterized using the Port-a-Patch.

References

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Methods

Cells

HEK293 cells stably expressing α5β3γ2 $GABA_A$ receptors were kindly provided by Merck Millipore.

Cell culture

HEK293 cells stably expressing α5β3γ2 $GABA_A$ receptors were cultured using standard culture conditions for the Port-a-Patch.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Port-a-Patch. Currents were elicited by 2 s perfusion of GABA at different concentrations. A continuous holding potential of -80 mV was used. Bicuculline was pre-incubated for 2 mins prior to co-application with GABA.

Nanion Technologies GmbH
Ganghoferstr. 70A
80339 Munich, Germany

phone +49 89 219 095-0
fax +49 89 218997960
www.nanion.de • info@nanion.de

nan]i[on