Application Note

Channel: $GABA_A (\alpha_1\beta_2\gamma_2)$
Cells: HEK293
Tools: Patchliner©

Investigating the pharmacology of $GABA_A (\alpha_1\beta_2\gamma_2)$ receptors expressed in HEK293 cells.

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Summary

Using the Patchliner platform, the pharmacology of γ-amino butyric acid (GABA), β-alanine and bicuculline were investigated on $GABA_A$-receptors expressed in HEK293 cells.
The $GABA_A$ receptor family is the most important class of inhibitory ion channels involved in synaptic transmission. $GABA_A$ receptors are selectively permeable to monovalent anions. They constitute an important therapeutic target for drugs affecting anxiety, sleep and muscle relaxation.
In this study, the cells were exposed to compound for 30 s, followed by a 60 s wash step. Solution exchange around the cell was fast, in the order of 50 ms for saturating concentrations, see Fig. 1. Complete and rapid switching is important when investigating ligand gated ion channels, since the response is often very fast and most receptors desensitize. For rapidly desensitizing ion channels, fast compound application is crucial, so that the entire ion channel population is exposed to maximum concentration before entering the desensitized state. Desensitization and recovery kinetics vary from milliseconds to tens of minutes, depending on receptor type and subunit composition as well as exposure time and/or compound concentration.
Recordings were made in the whole-cell configuration, using the Patchliner (4 amplifier channels). Expected pharmacology was obtained for the investigated compounds.

Results

GABA was rapidly added to the cells with a total exposure time of 30 s. Wash intervals with control solution were 60 s. Figure 1 shows the concentration dependent activation of $GABA_A$-receptors by application of increasing concentrations of GABA (0.3, 1, 3, 10, 30, 100 µM). Peak current amplitudes were normalized against the first saturating response (*Figure 2*). The EC_{50} was determined as 2.7 ± 0.5 µM from the Hill plot. The error bars show the standard error of the mean.

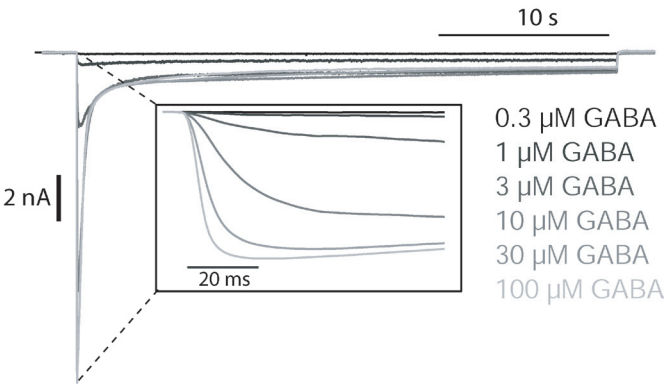

***Figure 1**: Whole-cell currents evoked by GABA-applications (increasing concentrations) for the determination of the EC_{50}. Application is fast, well below 20 ms for the higher concentrations (insert).*

Compound application is fast. In these experiments it ranged from 50 ms to 100 ms (mean 78 ms) for the application of 10 µM of GABA, and was faster for concentrations higher than 10 µM, see insert *Figure 1*.

The pharmacology of the agonist β-Alanine was also investigated. The exposure time to β-Alanine was 30 s followed by a 60 s wash step. The peak current amplitudes were normalized against the first saturating response and plotted against log concentration (Figure 3). The EC_{50} value was determined as 670 ± 70 µM. As expected, the potency of β-Alanine is at least two orders of magnitude lower than that of GABA.

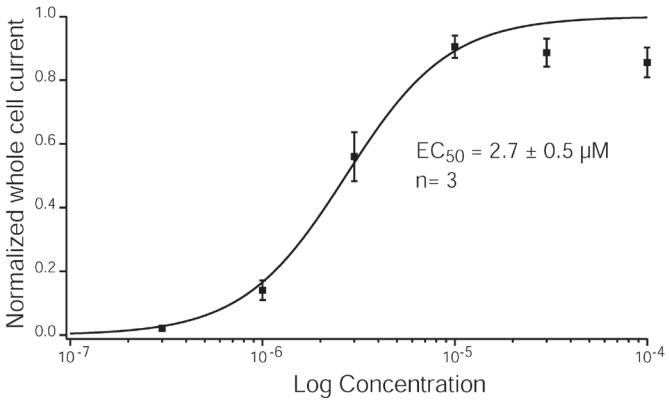

Figure 2: *The EC_{50} value for GABA was determined by plotting the normalized peak current amplitudes against concentration (log scale). The obtained value corresponds well with the literature.*

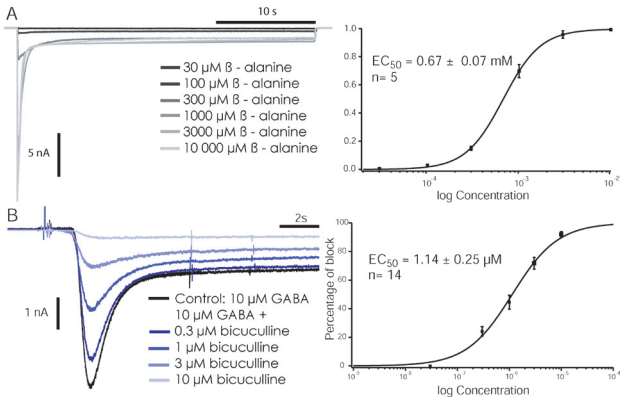

Figure 3: *(A) The EC_{50} for β-Alanine was determined by the application of increasing concentrations of β-Alanine with intermittent wash steps. (B)The IC_{50} for bicuculline was determined by the appliation of control (10 µM GABA) followed by co-application of 10 µM GABA with increasing concentrations of bicuculline.*

The potency of the anatgonist bicuclline was investigated. Increasing concentrations (0.3, 1, 3, 10 µM) of bicuculline were co-applied with 10 µM GABA, for determination of the IC_{50} value. The values for the peak amplitudes were normalized against the control (10 µM GABA - *Figure 3*). The IC_{50} value was determined from the Hill plot as 1.14 µM, which corresponds well to the literature (1 -3 µM).

References

1. Hille, B., Ion channels of Exitable Membranes, 2001, Third Edition, Sinauer Press.
2. Lu, J., Greco MA., 2006, Sleep circuitry and the hypnotic mechanism of $GABA_A$ drugs, J. Clin. Sleep Med. 15:19-26
3. Cosette, P., Lidong, L., Brisebois, K., et al. 2002, Mutation of GABA1 in autosomal dominant form of juvenile myoclonic epilepsy, Nature genetics, 31:184-189
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Methods

Cells
HEK293 cells stably expressing $GABA_A$ were used.

Cell culture
Cells were cultured and harvested according to Nanion's standard cell culture protocol.

Electrophysiology
Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner©. Cells were clamped to a holding potential of -80 mV. Compounds solutions were diluted in external recording solution, prepared daily from frozen stocks.

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