



Application Note

Channel: **GABA_A($\alpha_1, \beta_2, \gamma_2$)**
 Cells: **HEK293**
 Tools: **Patchliner[®]**

Rapid agonist applications in precise intervals using the Patchliner[®]

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Summary

HEK-cells expressing GABA_A-receptors were investigated with the Patchliner using a stacked application approach for rapid administration of compounds to the patch-clamped cells.

The GABA receptor family is the most important class of inhibitory ion channels involved in synaptic transmission, and are selectively permeable to monovalent anions. They constitute an important therapeutic area for drugs affecting anxiety, sleep and muscle relaxation.

As with most ligand gated ion channels, GABA_A exhibit receptor desensitization, which is a common phenomenon for ligand gated ion channels. Desensitization can be either exposure time dependent or concentration dependent, or both. Desensitization and recovery kinetics varies from milliseconds to tens of minutes, all depending on receptor type and subunit composition. For rapidly desensitizing ion channels, it is important that compound application is fast, so that the entire ion channel population is exposed to maximum concentration before entering the desensitized state.

Exposure time and application intervals are important factors affecting desensitization and recovery from desensitization, to minimize deleterious effects or receptor desensitization.

In the experiments presented here, compounds were added to the cells with accurate timing. In addition, a method for brief compound application was developed, allowing 1 s pulses of compound to the patch

clamped cells. In this way, receptor desensitization can be minimized, and because of the timed exposures, effects of desensitization can be accounted and corrected for.

Results

A method for stacked application was developed. Here, buffer solution was aspirated, directly followed by a plug of compound. In this way, the cell was exposed to GABA for 1 s, at an interval of 90 s. Figure 1 shows the replay window of HEKA's Patchmaster software, showing the application of 1, 3 and 10 μ M of GABA to four patch clamped cells.

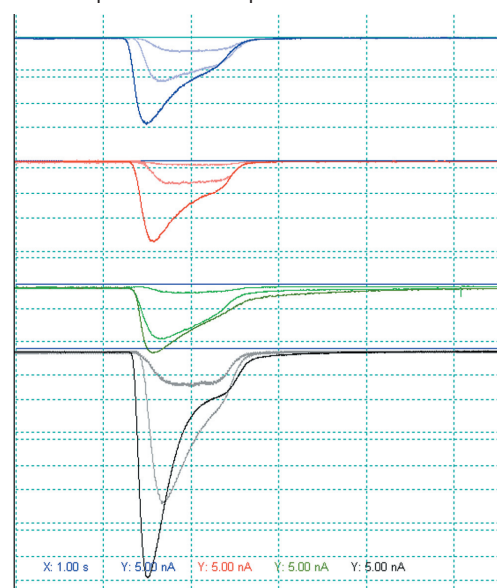


Figure 1: Replay window from HEKA, showing the application of GABA (1, 3 and 10 μ M) to four cells.

The current responses obtained from the stacked applications were compared to normal compound application, using the same concentrations of GABA. Here, application time was set to 20 s, with an application interval of 60 s.

The long applications were followed by stacked applications using the same concentrations, and as seen from the peak current responses, application time and wash time are acceptable for avoiding receptor desensitization.

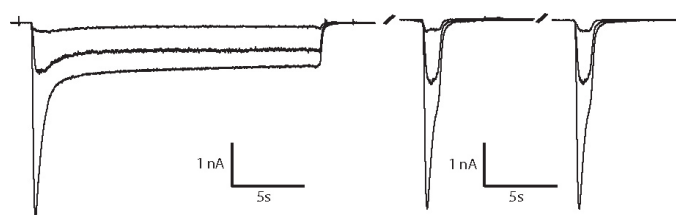


Figure 2: Comparison of long applications of GABA and the stacked application protocol. GABA (1, 3, 10 μ M) was applied in timed intervals.

The effect of the antagonist bicuculline was investigated.

In these experiments, shown in Figure 3, four cells were stimulated 3 times with control applications of GABA, 3 μ M. Control applications were followed by three co-applications of bicuculline and GABA (10 μ M, 3 μ M respectively). Peak current amplitudes were plotted against time, showing fairly consistent whole-cell responses over an extended time period, and that the block is reproducible.

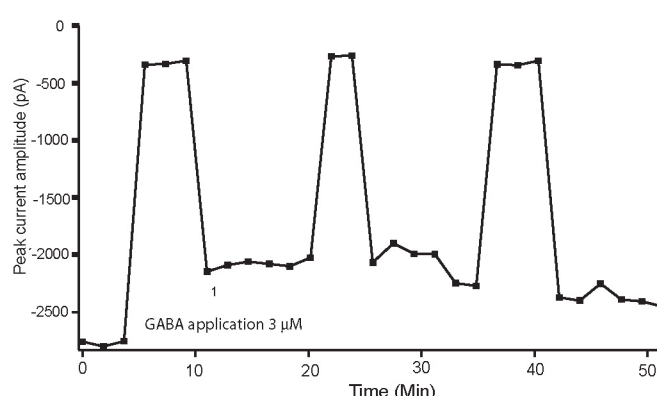
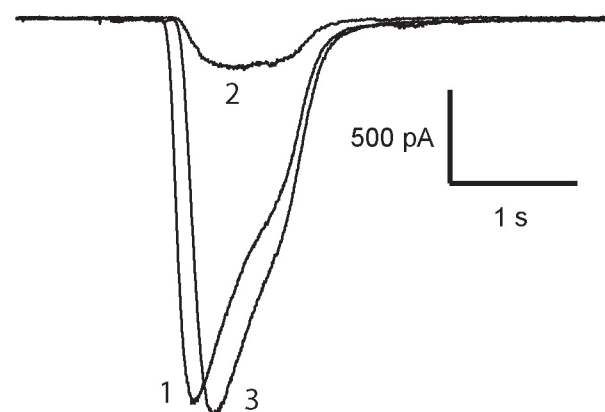


Figure 3: Investigation of bicuculline block. Bicuculline was co-applied using the stacked solution protocol (2), and compared to control applications of GABA (1 and 3). Peak current amplitudes were plotted over time as seen in the lower panel.

References

1. Hille, B., Ion channels of Excitable 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

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.