

## Modulators for glycine receptors investigated using the Patchliner<sup>®</sup>

The electrophysiology team at Nanion Technologies GmbH, Munich. Data reproduced by courtesy of AstraZeneca. Cells and drugs were kindly provided by AstraZeneca, Sweden.

### Summary

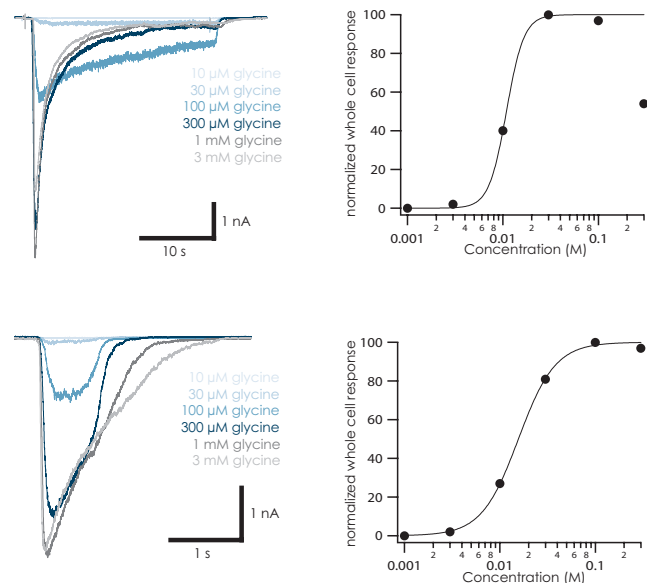
The Patchliner<sup>®</sup> was used to study the pharmacology of the hGlyR $\alpha$ 1 receptor expressed in a mouse fibroblast cell line (L-tk). The Patchliner<sup>®</sup> allows for fast application of drugs (<50 ms) with precisely controlled application intervals and wash times. These are important experimental parameters when investigating ligand gated ion channels and their effectors since many ligand gated ion channels rapidly desensitize. Kinetics and level of desensitization are determined by ligand concentration, exposure time, or both. Because of the Patchliner<sup>®</sup>'s rapid solution exchange combined with brief drug exposure capabilities and timed application intervals, the deleterious effect caused by receptor desensitization can be minimized and corrected for.

Here we show the use of Nanion's Patchliner<sup>®</sup> for long (22 s) and short (1 s) application of glycine to patch clamped whole-cells. For the short applications of compound, a stacked solution application protocol was used. Specifically, two zones of solutions were aspirated into the pipette used for administration. When applied to the cell, this results in exposure first to the agonist zone followed by a rapid wash out after 1 s of drug application. In all the protocols used, pre-incubation of modulators is possible, as well as washout in the presence of a modulator.

### Results

The pharmacology of glycine was investigated on GlyR $\alpha$ 1 using two different application strategies. Long and short application times were used to obtain dose response

curves and these are compared in Fig. 1. Concentrations ranging from 10 – 3000  $\mu$ M glycine were used.



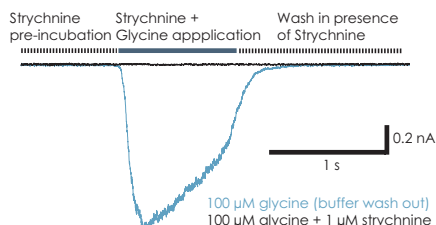
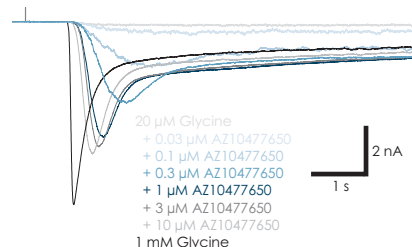
**Figure 1:**

Dose response of glycine. Top: Six concentrations of glycine (10, 30, 100, 300, 1000, 3000  $\mu$ M) were applied to the cells with intermittent wash steps. Four cells were simultaneously recorded,  $V_{\text{hold}} = -80$  mV. Cells were exposed to glycine for 22 s followed by a 60 s wash step. Bottom: A stacked application protocol was also used for 1 s application of glycine to the cells. The same concentrations were used,  $V_{\text{hold}} = -80$  mV.

# Application Note

Saturating whole-cell current responses were obtained between 300 - 1000  $\mu\text{M}$  and the  $\text{EC}_{50}$  was determined to be 127  $\mu\text{M}$ . Application of 3000  $\mu\text{M}$  glycine showed a substantial desensitization and the peak current response did not reach the amplitudes achieved with 300 and 1000  $\mu\text{M}$  glycine. When the stacked application protocol was used, the cells were exposed to glycine for 1 s followed by a 60 s wash step. Whole-cell currents elicited by 10 – 3000  $\mu\text{M}$  glycine are shown in the lower panel of Figure 2. As before, currents start to saturate at glycine concentrations above 300  $\mu\text{M}$ . However, here the peak current amplitudes evoked by 3000  $\mu\text{M}$  of glycine clearly reach saturating concentrations, which is not the case for the long applications. The  $\text{EC}_{50}$ , determined from the Hill plot, was 157  $\mu\text{M}$ . This indicates that receptor desensitization is exposure-time dependent. It is also clear that desensitization kinetics are concentration dependent, which is evident from both long and short agonist exposures.

Positive and negative modulators have been investigated using the Patchliner<sup>®</sup>. In the top panel of Figure 2 whole-cell traces are shown, showing potentiation of glycine responses. Here, first two control applications of glycine (20  $\mu\text{M}$ ) alone were made followed by co-administration with increasing concentrations of the positive modulator.



**Figure 2:**

The effect of positive and negative modulators acting on the GlyR $\alpha$ s was investigated. Specifically, a positive modulator was co-administered in increasing concentrations with 20  $\mu\text{M}$  of glycine to investigate the  $\text{EC}_{50}$  and degree of potentiation. In addition, the antagonist strychnine was investigated. A pre-incubation step was used.

As a final control, 1000  $\mu\text{M}$  of glycine was applied. The expected  $\text{EC}_{50}$  value and levels of potentiation were obtained.

The lower panel shows block of the hGlyR $\alpha$ 1-mediated current by the competitive antagonist strychnine. Two control applications of glycine were made, before and after the strychnine application. Currents were not regained after the block, so the second control is not shown. Strychnine (1  $\mu\text{M}$ ) was pre-incubated for 20 s, followed by the co-administration with 100  $\mu\text{M}$  glycine. Stacked applications were used to apply the compounds.

## References

1. Lynch JW, Callister RJ. 2006. Glycine receptors: a new therapeutic target in pain pathways. *Curr Opin Investig Drugs*. 7:48-53.
2. Barry PH, Lynch JW. 2005. Ligand-gated channels. *Trans Nanobioscience*. 4:70-80.

## Methods

### Cells

L-tk cells stably expressing hGlyR $\alpha$ 1 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<sup>®</sup>. Cells were held at a holding potential of -80 mV. For the short exposure times, solutions were stacked in the robotic pipettor. First, wash solution was aspirated followed by aspiration of the agonist-containing solution. Wash solutions contained the antagonist in the strychnine experiments. The cells were pre-incubated in strychnine before co-application with glycine.