Subunit-specific inhibition of recombinantly expressed glycine receptors by ginkgolides and bilobalide

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Extracts from the ginkgo biloba tree have been used in traditional Chinese medicine for centuries. Major active components of these extracts include the ginkgolides A, B and C (GA, GB, GC) and bilobalide (BB). GA, GB and GC are terpene trilactones which differ only in the number and placement of their hydroxyl groups. They share some structural similarity with BB and with the known glycine receptor Cl^- channel (GlyR) inhibitor, picrotoxin. The ginkgo compounds have recently been shown to have potent inhibitory effects on GlyRs endogenously expressed in cultured central neurons (Kondratskaya *et al.*, 2001; Ivic *et al.*, 2003). Their use- and voltage-dependence suggest they may be pore-blockers (Kondratskaya *et al.*, 2001; Ivic *et al.*, 2003). The aim of the present study was to investigate the specificity of these compounds for recombinantly expressed α1, α2, α1β and α2β GlyRs. Their use-dependence, voltage-dependence and agonist concentration dependence of inhibition were also examined.

HEK293 cells were transfected with GlyR cDNAs by the calcium phosphate precipitation protocol. The α and β subunits were co-expressed in a 1:10 ratio. The transfection solution was removed after 24 h and glycine-gated currents were recorded by whole-cell recording over the following 24−72 h. Heteromeric GlyRs were identified by GFP fluorescence and by their reduced sensitivity to picrotoxin inhibition.

In homomeric α2 GlyRs, inhibition by GA, GB and GC was more pronounced at positive voltages whilst BB showed no significant voltage-dependence. Lower concentrations of glycine markedly increased the inhibitory potency of BB, whereas glycine concentration changes had no significant effect on the degree of inhibition by GA, GB and GC. All four extracts showed use- dependence with no inhibition observed in the absence of glycine. As with picrotoxin, the potency of BB inhibition was drastically reduced upon co-expression of the β subunit with either the α1 or α2 subunits. On the contrary, co-expression of the β subunit with either the α1 or α2 subunits caused a significantly increased sensitivity to GB and GC. The sensitivity to GA was significantly increased in the α2β relative to the α2 GlyR, but was not significantly changed in the α1β relative to the α1 GlyR. The α1 subunit mutation T6′F abolished inhibition by all compounds.

The use-dependence, voltage-dependence and the sensitivity to the pore-lining T6′F mutation, all suggest that the 4 tested ginkgo biloba extracts bind at a site in the GlyR pore. The results however indicate a different mechanism of inhibition by BB compared to that of GA, GB and GC. BB inhibition of the GlyR appears to mimic the effects of picrotoxin, despite these compounds sharing little structural similarity. The subunit-specificity of these compounds may be of use in defining the subunit composition of native neuronal GlyRs. Further investigations are required to examine the molecular basis of the observed differences in their mechanisms and subunit-specificity of action.

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Ivic L., Sands, T.T., Fishkin, N., Nakanishi, K., Kriegstein, A.R. & Stromgaard, K. (2003) *Journal of Biological Chemistry* **278**, 49279-49285.