Mixed antagonistic effects of the ginkgolides at recombinant human ρ1 $GABA_C$ receptors

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Ginkgolides A, B and C are diterpene lactones found in the leaves of the Ginkgo biloba tree. They are known to be non-competitive antagonists at $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors (Huang *et al.*, 2004) and glycine receptors (Hawthorne *et al.*, 2006; Heads *et al.*, 2008). These receptors are both anion-selective channels of the pentameric ligand-gated ion channel family. The subunits that make up these receptors share a similar topology, with a large extracellular N-terminus that forms the ligand binding site, four transmembrane domains (M1-M4) and a short extracellular C-terminus. The M2 domain from each subunit forms the channel pore. Evidence suggests that ginkgolides bind within the channel pore by interacting with M2 residues (Hawthorne *et al.*, 2006; Heads *et al.*, 2008).

The effects of ginkgolides A, B and C were examined on recombinant human ρ1 $GABA_C$ receptors expressed in Xenopus oocytes. Whole-cell currents were recorded using standard two-microelectrode voltage-clamp methods. Oocytes were clamped at –60 mV and continuously superfused with ND96 buffer. GABA concentration-response curves were compiled from a range of GABA concentrations (0.01 − 100 μM) applied alone or co-applied with a fixed concentration of ginkgolide (10 μM, 30 μM or 100 μM) in ND96 buffer. The GABA EC_{50} value was 1.02 ± 0.05 μM (n=15) and with increasing ginkgolide concentration there was an approximately parallel shift to the right of the curves and a decrease in the maximum response. Ginkgolide A (n=5) was the least potent of the three compounds, with a 1.9-fold, 2.3-fold and 2.5-fold increase in the EC_{50} value in the presence of 10, 30 and 100 μM respectively, with a maximum response of 86 ± 1% at 100 μM. Ginkgolide B (n=6) was the most potent, with 3.2-fold, 4.7-fold and 7.5-fold increases in the EC_{50} and a maximum response of 70 ± 2% at 100 μM. Ginkgolide C (n=4) was similar to ginkgolide B, with 3.8-fold, 6-fold and 5.3-fold increases in the EC_{50} and a maximum response of 73 ± 3% at 100 μM. Inhibition curves were compiled from a range of ginkgolide concentrations co-applied with 0.5 μM (~EC_{15} GABA), 1.2 μM (~EC_{50}), 3 μM (~EC_{80}) or 10 μM (~EC_{100}) test concentrations of GABA in ND96 buffer. Each of the ginkgolides A, B and C exhibited increased IC_{50} values with increasing GABA concentrations. These results are indicative of a mixed-antagonism effect and in an attempt to explain these results a simple allosteric mechanism of inhibition was fitted to the data. With such an allosteric kinetic scheme, the rightward shift in the concentration response curves is accounted for by an increase in the GABA equilibrium dissociation constant (decrease in affinity) in the presence of ginkgolides, and a decrease in the equilibrium for the channel opening reaction (decrease in open probability) accounts for the decrease in maximum response. However, not all characteristics of the inhibition curves were adequately predicted by such a scheme. The difference in antagonism at ρ1 $GABA_C$ receptors compared to $GABA_A$ and glycine receptors may be due to subtle differences in the amino acid residues located at the bottom of the channel pore, where the ginkgolides are thought to bind.

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