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Ginsenoside Rg_3 Blocks the Open State of Brain Na^+ Channel

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Recent study showed that ginsenosides, active ingredients of *Panax* ginseng, inhibit brain-type Na^+ channel activity. However, the molecular mechanisms involved in ginsenoside-induced Na^+ channel regulation have not yet been determined precisely. To provide answers to these questions, we investigated the effect of ginsenoside Rg_3 (Rg_3) on inward peak Na+ current (I_{Na}) expressed in *Xenopus* oocytes after injection of cRNAs encoding rat brain Navl.2 α and β1 subunits using two-microelectrode voltage clamp technique. In wild-type Na^+ channel, Rg_3 induced tonic and use-dependent inhibitions of I_{Na} at low and high stimulations. The tonic inhibition of I_{Na} by Rg_3 was reversible, voltage-, and dose-dependent manner. The IC_{50} on wild-type Na+ channel was 36.1 ± 6.5 μM. Using site-directed mutagenesis method on sodium channel a subunit, we examined the Rg_3 block in mutants of pore regions of domain II, TTX binding sites and inactivation cluster, IFMQ3 mutant which lacks fast inactivation, and found that mutations of pore region and TTX binding sites of Na+ channel did not affect Rg_3 block. IFMQ3 mutation abolished Rg_3 block in peak but not in non-inactivating Na^+ current, indicating that IFMQ3 mutation directly affects Rg_3 block on I_{Na}. The IC_{50} on IFMQ3 mutant peak Na+ current was 129.6 ± 14.3 μM. The IC_{50} value of IFMQ3 channel was 3.6-fold higher than that of the wild-type Na+ channel. These results suggest that Rg_3 is an open Na+channel blocker and that the hydrophobic cluster is not only involved in Na^+ channel inactivation but also related with Rg_3-induced Na^+ channel regulation.