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SIKSTUS, SYLVIA: AA29504 Modulation of Radioligand Binding to  $\alpha\beta\gamma 2$  and  $\alpha\beta\delta$  GABA<sub>A</sub> Receptors

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In this study, we researched the effects of an allosteric modulator on GABA<sub>A</sub> receptors. Allosteric modulator is a compound which indirectly modulates the effects of ligands at receptors. The allosteric modulator used in this study was [2-amino-4-(2,4,6-trimethylbenzylamino)-phenyl]-carbamic acid ethyl ester (AA29504) that has been suggested to be selective for  $\delta$  subunit-containing GABA<sub>A</sub> receptors. The main focus was to compare the effects of AA29504 on  $\gamma 2$  and  $\delta$  subunit-containing GABA<sub>A</sub> receptors.  $\delta$ -containing receptors are located extrasynaptically and therefore modulate tonic inhibition in the brain, while  $\gamma 2$ -containing are located in both synaptic and extrasynaptic sites.

We studied the effect of AA29504 using radioligand binding assays. We used forebrains of wild-type (WT) and GABA<sub>A</sub>  $\delta$  subunit knockout ( $\delta$ KO) mice, and recombinant receptors expressed in human embryonic kidney 293 cell line. We measured the effect of AA29504 on binding of both [<sup>3</sup>H]GABA and [<sup>3</sup>H]muscimol, and on the association and dissociation of [<sup>3</sup>H]muscimol. We also measured the effect of AA29504 on GABA and THIP induced displacement of [<sup>3</sup>H]EBOB binding.

AA29504 stimulated [<sup>3</sup>H]GABA and [<sup>3</sup>H]muscimol binding and potentiated GABA- and THIP-induced displacement of [<sup>3</sup>H]EBOB binding to both WT and  $\delta$ KO mouse forebrain GABA<sub>A</sub> receptors. However, it had no effect on GABA-agonist binding to recombinant  $\alpha\beta\delta$  receptors while strongly stimulating it to  $\alpha\beta\gamma 2$  receptors. Our results question the hypothesis of AA29504 being a  $\delta$  subunit-specific allosteric modulator.

Key words: GABA, muscimol, AA29504, GABA<sub>A</sub> receptor, EBOB, allosteric modulation