Effects of neuroactive steroids on the recombinant GABA<sub>A</sub> receptor in *Xenopus* oocyte

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Abstract

Introduction: Neuroactive steroids represent a class of both synthetic and naturally occurring steroids that have an effect on neural function. In addition to classical genomic mechanism by the hormones progesterone, deoxycorticosterone and testosterone, the 3α-OH metabolite of these hormones enhance GABA<sub>A</sub> receptor through rapid non-genomic mechanism. The site(s) of action of these neuroactive steroids namely 3α-OH-5α-pregn-20 one (3α5αP), (3α,5α)-3,21-deoxycorticosterone(3α5α-THDOC) and 5α androstan-3α,17β-diol on GABA<sub>A</sub> receptor are distinct from that of benzodiazepines and barbiturate binding sites. The modulation site(s) has a well-defined structure activity relationship with a 3α-hydroxy and a 20-keone configuration in the pregnane molecule required for agonistic action. Pregnenolone sulfate is a non-competitive GABA<sub>A</sub> receptor antagonist and inhibit GABA activated Cl⁻ current in an activation dependant manner. 3β-hydroxy A-ring reduced pregnane steroids are also GABA<sub>A</sub> receptor antagonist and inhibit GABA<sub>A</sub> receptor function and its potentiation induced by their 3α,diesteromers in a non-competitive manner.

Aim: The aim was to investigate if the effect of GABA, pentobarbital antagonism by bicuculline and if the effect of GABA agonist and antagonist neuroactive steroids including pregnenolone sulfate is dependant on the α-subunit of GABA<sub>A</sub> receptor. Furthermore, the studies aimed at investigating the binding site of pregnenolone sulfate and if its effect is dependant on γ-subunit. In addition, the inhibitory effect of pregnenolone sulfate and 3β-hydroxy steroids has been characterized. We also wanted to investigate if the neuroactive steroids effect vary between the human and rat recombinant α1β2γ2L receptors and between the long (L) and short (S) variants of γ2-subunit.

Method: Experiments were performed by the two electrodes voltage-clamp technique using oocytes of Xenopus laevis expressed with recombinant GABA<sub>A</sub> receptors containing α1, α4 or α5, β2, γ2L and γ2S-subunits.

Results: There was no difference between the α1, α4 and α5-containing subunits regarding GABA and pentobarbital inhibition by bicuculline. GABA-activated current in the binary α9 is potent then that of ternary α9γ receptor. Unlike Zn<sup>2+</sup> effect, inhibition by pregnenolone sulfate on the GABA<sub>A</sub> receptor is not dependant on the γ-subunit. It is likely that the 2' residue closest to the N-terminus of the protein at M3 helix on both α1 and β2 subunit are critical to the inhibitory actions of PS and the function of Cl⁻ channels. Point mutation at M3 helix of the β2-subunit (B2A252S) can dramatically reduce the inhibitory effect of PS on the GABA<sub>A</sub> receptors without affecting the inhibitory properties of 3β-hydroxysteroids. Agonist and antagonist steroids also varied in their efficacy between the human and rat α1β2γ2L receptor. Neuroactive steroids also showed difference between human γ2L and γ2S-containing receptor.

Conclusions: GABA and pentobarbital antagonism by bicuculline is not dependant on α-subunit. Pregnenolone sulfate binding site is different from that of Zn<sup>2+</sup>. 3β-hydroxysteroids and pregnenolone sulfate inhibit GABA<sub>A</sub> receptor through different mechanism. Neuroactive steroids also differ between species and between the long and short variant of γ-subunit.

Key words GABA, GABA<sub>A</sub> receptor, neuroactive steroids