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Effects of neuroactive steroids on the recombinant $GABA_A$ receptor in *Xenopus* oocyte

av

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Effects of neuroactive steroids on the recombinant $GABA_A$ 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 $GABA_A$ receptor through rapid non-genomic mechanism. The site(s) of action of these neuroactive steroids namely 3α-OH-5α-pregnan-20 one (3α5αP), (3α,5α)-3,21-deoxycorticosterone(3α5α-THDOC) and 5α androstane-3α,17β-diol on $GABA_A$ 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-ketone configuration in the pregnane molecule required for agonistic action. Pregnenolone sulfate is a noncompetitive $GABA_A$ receptor antagonist and inhibit GABA activated Cl^- current in an activation dependant manner. 3β-hydroxy A-ring reduced pregnane steroids are also $GABA_A$ receptor antagonist and inhibit $GABA_A$ receptor function and its potentiation induced by their 3α-diesteromers in a noncompetitive 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-subunits of $GABA_A$ receptor. Furthermore, the studies aimed at investigating the binding site of pregnenolone sulfate and if its effect is dependent 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 $GABA_A$ 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 αβ was potent than that of ternary αβγ receptor. Unlike Zn^{2+} effect, inhibition by pregnenolone sulfate on the $GABA_A$ receptor is not dependant on the γ-subunit. It is likely that the 2' residue closest to the N-terminus of the protein at M_2 helix on both α1 and β2 subunit are critical to the inhibitory actions of PS and the function of Cl^- channels. Point mutation at M_2 helix of the β2-subunit (β2A252S) can dramatically reduce the inhibitory effect of PS on the $GABA_A$ 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^{2+}. 3β-hydroxysteroids and pregnenolone sulfate inhibit $GABA_A$ receptor through different mechanism. Neuroactive steroids also differ between species and between the *long* and *short* variant of γ- subunit.

Key words GABA, $GABA_A$ receptor, neuroactive steroids