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Brain Region-Specific Effects of Neuroactive Steroids on the Affinity and Density of the GABA-Binding Site

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Summary: The allosteric regulation of specific [^{3}H]-muscimol binding by neuroactive steroids to the GABA-binding sites of membrane fractions prepared from five different brain areas was characterized in order to elucidate if the regionally variable subunit composition of $GABA_A$ receptors is reflected in the responsiveness of the GABA binding site to neurosteroid modulatory effects. At a final concentration of 1μM progesterone and its metabolite 3-α-hydroxy-5-α-pregnane-20-one (HPO) reduced the affinity in hippocampus (HIP), enhanced it in medulla (MED) and did not affect it in cerebellum (CER). However, there are differences in potency of these two steroids between frontal cortex (FC) and hypothalamus (HYP), since the affinity was enhanced in FC only by progesterone and in HYP only by HPO. While the magnitude of progesterone-induced alterations in affinity were similar in FC, MED and HIP, HPO affected the affinity significantly stronger in HIP than in HYP and MED. Concerning the density of the binding sites progesterone exerted no significant modulatory effect in contrast to HPO which increased the number of binding sites (B_{max}) in all five brain areas investigated. However, the enhancements in B_{max} were regionally different. The HIP reached the maximal increase of B_{max}, followed by FC and MED. The smallest enhancement was found in CER, followed by HYP. Neurosteroidal activity exhibited also THDOC and alphaxalone, the synthetic HPO analogue. A significant different potency of THDOC was found in FC versus CER, whereas alphaxalone did not display regionally different efficacy. The present investigation shows that steroidal modulation of the GABA binding site highly depends on the kind of steroid investigated and differs between brain areas. These findings give evidence that certain endogenous steroid metabolites are potent and highly selective modulators of the $GABA_A$ receptor thus supporting a physiologic role of these steroids in the regulation of brain excitability.

Key terms: $GABA_A$ receptor, rat brain areas, progesterone, 3-α-hydroxy-5-α-pregnane-20-one, 5-α-pregnane-3-α,21-diol-20-one, alphaxalone.

The $GABA_A$ receptor is assembled from various combinations of different subunits as demonstrated by the isolation of cDNAs encoding six α-subunits[1,2], three β-subunits[3], two γ-subunits[4] and one δ-subunit. Furthermore, the distribution of the corresponding mRNAs differs between brain regions and even between cell types in those regions[5] suggesting the occurrence of region-specific molecularly and functionally distinct receptor subtypes[6,7]. These receptor isoforms contain several binding sites for pharmacologically specific agents which modulate GABA receptor chloride channel function[8]. In particular, benzodiazepines, barbiturates, and picrotoxin-like convulsants bind to receptor sites directly located on

Abbreviations:
GABA: γ-aminobutyric acid; HPO: 3-α-hydroxy-5-α-pregnane-20-one; THDOC: 5-α-pregnane-3-α,21-diol-20-one; alphaxalone: 5-α-pregnane-3-α-hydroxy-11,20-dione; PBCS: phosphate-buffered chloride solution; K_D: equilibrium dissociation constant; B_{max}: maximal binding capacity; POPSO: disodium-piperazine-*N,N'*-bis[2-hydroxypropanesulfinic acid].