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Neuroactive Steroid Actions at the $GABA_A$ Receptor

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Neuroactive steroids are a new class of steroids that do not interact with any of the classical cytosolic hormonal steroid receptors. The most well-documented examples are those that interact with the γ-aminobutyric $acid_A$ ($GABA_A$) receptor/chloride channel complex in the central nervous system. The $GABA_A$ receptors are known to contain allosteric modulatory sites for therapeutically useful drugs such as benzodiazepines (BZs) and barbiturates. The interaction of neuroactive steroids with the $GABA_A$ receptor is specific to a site on the receptor complex distinct from the benzodiazepine and barbiturate modulatory sites. Neuroactive steroids exist endogenously; the examples are metabolites of progesterone and deoxycorticosterone, 3α-hydroxy-5α-pregnane-20-one, and 5α-pregnane-3α, 21α-dihydroxy-20-one, respectively, and their 5β stereoisomers. The $GABA_A$ receptor agonist-like effects that these neuroactive steroids produce *in vivo* are similar, but not identical, to those of BZs and barbiturates. Representatives of all three classes of modulators are active as sedative-hypnotics, anticonvulsants, and anxiolytics in animal models. Because of the heterogeneity of $GABA_A$ receptors and their differential distribution in the brain, dissimilar *in vivo* pharmacological profiles displayed by BZs, barbiturates and neuroactive steroids are not surprising. Studies of neuroactive steroid interactions with the $GABA_A$ receptor revealed a unique subset of these steroids that modulate the receptor with limited efficacy. Another endogenously occurring progesterone metabolite, 5α-pregnane-3α,20α-diol, is an example of this subset of neuroactive steroids. At present, it is not clear whether the observed limited efficacy is due to receptor subtype selectivity, partial agonist activity or both. Nevertheless, data from recombinantly expressed $GABA_A$ receptors suggests that receptor subunit composition influences the recognition properties of the neuroactive steroid site. Collectively, biochemical, pharmacological, and physiological studies support the existence of a novel binding site for neuroactive steroids on membrane-bound $GABA_A$ receptor complexes in the mammalian brain. The physiological role of this binding site and its endogenously occurring ligands may provide additional insight into how hormonal steroids may affect brain excitability in a non-genomic fashion.

Neuroactive steroids are a new class of steroids, some of which were recently found to interact specifically with a distinct binding site on the γ-aminobutyric $acid_A$ ($GABA_A$) receptor complex in the central nervous

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system (CNS) (for reviews, see Gee, 1988; Deutsch, Mastropaolo, and Hitri, 1992.) The $GABA_A$ receptor is known to mediate the action of the major inhibitory neurotransmitter, GABA, in the vertebrate brain. This receptor is a complex membrane-bound protein which is coupled to a Cl^- channel. Upon binding to the receptor, GABA facilitates the opening of the Cl^- channel resulting in hyperpolarization of the neuron. Therapeutically useful anticonvulsants, anxiolytics, and sedative-hypnotics such as the benzodiazepines (BZs) and barbiturates are known to act through their binding to the respective binding sites on the $GABA_A$ receptor complex (for review see Stephenson, 1988). These agents are positive $GABA_A$ allosteric modulators that increase the binding of GABA to the $GABA_A$ receptor. Conversely, negative $GABA_A$ receptor modulators such as some β-carbolines that decrease the binding of GABA to the $GABA_A$ receptor, are anxiogenic or convulsants (Braestrup, Schmiechen, Neff, Nielsen, and Petersen, 1982). The discovery of neuroactive steroids and their distinct binding site on the $GABA_A$ receptor complex raises the possibility that this new class of steroids may have therapeutic potential like other $GABA_A$ receptor ligands.

Neuroactive steroids are found endogenously. The most potent natural occurring neuroactive steroids are 3α-hydroxy-5α-pregnane-20-one (epiallopregnanolone; 3α,5α-P) and 3α,21-dihydroxy-5α-pregnane-20-one (tetrahydrodeoxycorticosterone; 5α-THDOC) which are metabolites of progesterone and deoxycorticosterone, respectively. These steroids are positive allosteric modulators of the $GABA_A$ receptor. In order to distinguish this novel class of steroids from classical hormonal steroids, the term epalons, an acronym for the word "epiallopregnanolone," was coined to define these neuroactive steroids, both natural and synthetic, that specifically interact with the $GABA_A$ receptor. A second class of neuroactive steroids represented by pregnenolone and dehydroepiandrosterone and their sulfate metabolites has been shown to be the negative modulator of the $GABA_A$ receptor (Carette and Poulain, 1984; Majewska and Schwartz, 1987). Although they have been reported to possess the behavioral, biochemical and electrophysiological profiles of epalons (Carette and Poulain, 1984; Mienville and Vicini, 1989; Majewska, Demirgoren, Spivak, and London, 1990; Demirgoren, Majewska, Spivak, and London, 1991), the site and mechanism of action of this second class of steroids remains elusive since there has been no clear structure–activity requirement observed so far. Evidence that led to the supposition that epalons interact with the $GABA_A$ receptor was provided by several detailed binding (Majewska, Harrison, Schwartz, Barker, and Paul, 1986; Gee, Bolger, Brinton, Coirini, and McEwen, 1988) and electrophysiological studies (Harrison, Majewska, Harrington, and Barker, 1987; Lambert, Peters, and Cottrell, 1987). 3α,5α-P, 5α-THDOC and other epalons were shown to allosterically modulate [^{35}S]-*t*-butylbicyclophosphorothionate ([^{35}S]

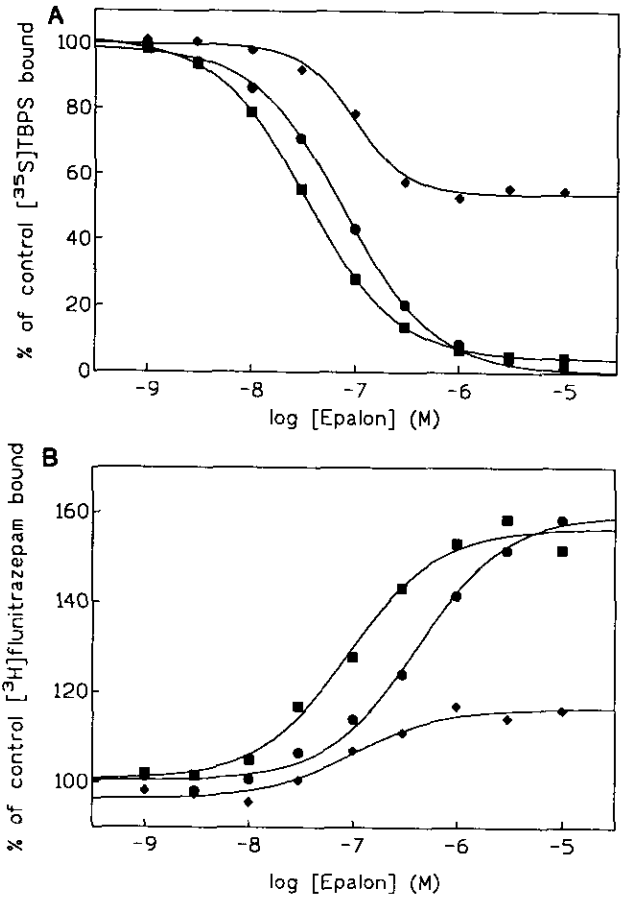

FIG. 1. Dose-dependent inhibition of [^{35}S]TBPS (2 n*M*) binding (A) or enhancement of [^{3}H]Flu (1 n*M*) binding (B) by 3α,5α-P (■), 5α-THDOC (●) or 5α-pregnan-3α,20α-diol (◆) in *rat* cerebral cortical P_2 homogenates. All assays were run at 25°C, 90 min in the presence of 5 μM (TBPS assay) or 1 μM (Flu assay) GABA. The $1C_{50}$ (concentration that produces half-maximal inhibition of [^{35}S]TBPS binding) values for 3α,5α-P, 5α-THDOC, and 5α-pregnan-3α,20α-diol are 33, 77, and 97 n*M*, respectively. The EC50 (concentration that produces half-maximal enhancement of [^{3}H]Flu binding) values for 3α,5α-P, 5α-THDOC and 5α-pregnan-3α,20α-diol are 92, 420, and 103 n*M*, respectively.

TBPS) (Fig. 1A) and [^{3}H]flunitrazepam ([^{3}H]Flu) binding (Fig. 1B) (Gee *et al.*, 1988). TBPS, a cage convulsant, is thought to bind to a site at or near the chloride channel, whereas Flu binds to the BZ site on the $GABA_A$ receptor complex. The affinities of [^{35}S]TBPS and [^{3}H]Flu for their sites are sensitive to conformational changes of the receptor induced by GABA binding. GABA agonists allosterically inhibit [^{35}S]TBPS and enhance [^{3}H]Flu binding, respectively. Epalons behave similarly to the GABA agonists as shown in Fig. 1. Studies measuring the ability of

epalons to enhance muscimol-sensitive $^{36}Cl^-$ uptake by rat cortical synaptoneurosomes (Morrow, Suzdak, and Paul, 1987) and GABA-induced current in neuronal cells (Harrison *et al.*, 1987; Lambert *et al.*, 1987), demonstrated their efficacy in functional assays. The site of action of the $GABA_A$ receptor complex for these activities was originally thought to overlap with those for barbiturates (Majewska *et al.*, 1986). However, subsequent electrophysiological and binding studies demonstrated that epalon-induced current was potentiated by barbiturates (Lambert *et al.*, 1987; Peters, Kirkness, Callachan, Lambert, and Turner, 1988) and that the dissociation of [^{35}S]TBPS binding induced by a saturating concentration of an epalon was accelerated by a maximally effective concentration of a barbiturate (Gee *et al.*, 1988). These studies argued against the original notion that epalons and barbiturates share a common site but rather supported the idea that epalons act on a site distinct from the barbiturate binding site.

An earlier study with limited numbers of epalons pointed to the importance of 3-α-hydroxylation for the activity (Gee *et al.*, 1988). Recent, extensive studies with hundreds of synthetic epalons further support the conclusion that 3α-hydroxylation is an absolute structural requirement for their activities whereas the modifications at various positions in the steroid nucleus will modify, but not necessarily eliminate, the compound's activity. Thus, all 3β-hydroxylated isomers tested so far are relatively inactive. In addition, these studies revealed the existence of epalons with different efficacies. The best example of limited efficacy epalons is yet another endogenously occurring neuroactive steroid, 5α-pregnane-3α,20α-diol, a further metabolite of 3α,5α-P. As shown in Fig. 1, 5α-pregnane-3α,20α-diol inhibited [^{35}S]TBPS binding or enhanced [^{3}H]Flu binding with lower efficacy than that achieved by of 3α,5α-P in rat cortical membrane preparations.

The limited efficacy of 5α-pregnane-3α,20α-diol was also demonstrated in $^{36}Cl^-$ uptake (Belelli and Gee, 1989) and electrophysiological studies (Belelli *et al.*, 1994). The studies on the modulation of GABA-stimulated $^{36}Cl^-$ uptake by cortical synaptoneurosomes suggested that the limited efficacy of 5α-pregnane-3α,20α-diol may be due to partial agonism (Belelli and Gee, 1989). However, recent detailed studies with recombinant $GABA_A$ receptor subunits point to the possibility that subtype selectivity may be responsible in part for its apparent limited efficacy (unpublished observation). Subtype selective allosteric BZ modulators of the $GABA_A$ receptor have been described.

Recent molecular cloning has revealed that the $GABA_A$ receptor, like the nicotinic-cholinergic receptor, is a hetero-oligomeric protein complex comprised of multiple subunits forming an intrinsic ion channel (for review see Olsen and Tobin, 1990). To date, at least 15 $GABA_A$ receptor subunits have been cloned that can be grouped into several families (α_{1-6}, β_{1-3},

TABLE 1

Epalons	*In vivo* pharmacology	References
5α-THDOC	Anxiolytic	Crawley *et al.* (1986)
5α-THDOC	*Sedative*-hypnotic	Mendelson *et al.* (1987)
3α,5α-P	Anticonvulsant	Belelli *et al.* (1989)
3α,5α-P/3α,5β-P	Anxiolytic	Bitran *et al.* (1991)
3α-hydroxy-5α-pregnan-11, 20-dione (alphaxalone)	Anxiolytic	Britton *et al.* (1991)
3α,5α-P	Anxiolytic	Wieland *et al.* (1991)

γ_{1-3}, δ, and ρ_{1-2}). Functional expression of these subunits in mammalian cell lines or Xenopus oocytes results in $GABA_A$ receptors with differential pharmacological characteristics (for review see Bureau and Olsen, 1990; Doble and Martin, 1992). Thus, well-known type I and type II benzodiazepine pharmacology detected by imidazopyridines such as zolpidem was revealed by the compound's selective affinity for receptors composed of α_1 versus α_2/α_3 subunits in combination with the β and γ subunits (Pritchett, Lüdens, and Seeburg, 1989; Pritchett and Seeburg, 1991). Subtype selective ligands are believed to have a better therapeutic profile and fewer undesirable side effects. Subtype selectivity of neuroactive steroids has not been clearly demonstrated. However, neuroactive steroids have been shown to respond differently in different regions of the CNS by binding assays (Lan and Gee, 1991) and autoradiographic studies (Sapp, Witte, Turner, Longoni, Kokka, and Olsen, 1992). Whether the observed regionally specific responses are due to subtype selectivity of neuroactive steroids awaits further study. Research involving the use of synthetic epalons and recombinant receptors for such studies is currently underway.

Understanding the mechanism of epalon action and the observation that the levels of endogenous epalons increase during stress, has led to speculation that they are endogenous anxiolytics produced in response to physiological stress (Purdy, Morrow, Moore, and Paul, 1991). The potential therapeutic utility of synthetic epalons has also been proposed. Taking 3α,5α-P and 5α-THDOC as prototypical examples, epalons have been shown to be anticonvulsants, anxiolytics and sedative-hypnotics in a variety of animal models as shown in Table 1. In addition, their activity profiles appeared to be similar but not identical to that of the BZs. Although this may have been anticipated based on receptor heterogeneity, our current studies are focused on the delineation of these *in vivo* differences examining the differential distribution of epalons versus BZ binding sites in various brain regions. Because both 3α,5α-P and 5α-THDOC occur endogenously, they have short metabolic half-lives and limited bioavailability. These properties hinder the development of these natural

occurring compounds as therapeutic agents. Therefore, synthetic epalons with similar therapeutic profiles but greater bioavailability, are being pursued. A clinical value for these compounds is foreseen; however, an assessment of the extent of the benefits of synthetic epalons over currently existing therapies awaits further study.

In summary, recent biochemical studies have revealed the existence of a unique modulatory site for neuroactive steroids on the $GABA_A$ receptor/Cl^- channel complex. This neuroactive steroid binding site does not recognize classical hormonal steroids nor do cytosolic steroid receptors interact with epalons. Consequently, the interaction of epalons with the $GABA_A$ receptor appears to be specific. Furthermore, this interaction follows stringent structural requirements. Because several epalons also occur endogenously and their levels increase during stress, there has been speculation that they may play a role as endogenous anxiolytics under stress conditions. Because of their action on the $GABA_A$ receptor complex, the therapeutic use of synthetic epalons as hypnotics, anticonvulsants, and anxiolytics is being explored.

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