Steroid and Barbiturate Modulation of the GABAa Receptor

Possible Mechanisms

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Abstract

This review describes the modulation of the GABAa receptor by steroid hormones and barbiturates and proposes guidelines for further research. Having examined the complex organization of the GABAa receptor complex and the multiple allosteric interactions between its drug and transmitter/modulator binding sites, the possibility that conformational changes of the receptor molecule may explain most of its characteristics is explored. On the basis of considerable evidence, we propose that the GABAa receptor may adopt as many as five different conformations. However, the heterogeneity of central GABAa receptor binding cannot only be explained by different configurations of a single protein. It also has been shown that different GABAa receptor subtypes exist within different brain regions. These receptor subtypes may differ from each other in their subunit composition. By describing the GABAa receptor as a macromolecular complex that may adopt different conformations and whose subunit composition may vary, it becomes possible to understand the molecular mechanisms by which steroid hormones modulate the receptor. This has led to two models of hormone actions. A first model addresses the direct effects that steroids exert on the GABAa receptor and predicts that steroid hormones may cause the conformation of the receptor complex to change between active and inactive states. A second model, which addresses the observed heterogeneity of GABAa receptor binding within the brain, suggests that steroid hormones may change the expression of the different subunits of the receptor complex by acting at the genomic level. This review complements other recent reviews describing the modulation of the GABAa receptor (Olsen and Venter, 1986; Gee, 1988).

Index Entries: GABAa receptor steroid and barbiturate modulation of; γ-aminobutyric acid$_a$ receptor, conformation of; chloride channel; benzodiazepine GABA interactions; estrogens, progestagens; corticosteroids; neurosteroids; pyrazolopyridines; picrotoxin; *t*-butylbicyclophosphorothionate (TBPS); five-state model; GABAa receptor subunits.

Introduction

Steroid hormones exert their effects on the brain by regulating neurotransmission and membrane excitability (McEwen et al., 1984). Recent research has shown that the GABAa receptor complex is a target for estrogens, progestagens, corticosteroids, and androgens. These hormones modulate the GABAa receptor either when administered in vivo or in vitro, and they do so via regulation of gene expression or by acting directly on the receptor complex itself (Majewska et al., 1986; Majewska, 1987b; Gee et al., 1987; Gee, 1988; O'Connor et al., 1988; Schumacher et al., 1989a,b). The latter mechanism allows steroid hormones to change the sensitivity of brain cells to the inhibitory effects of gamma-aminobutyric acid (GABA), even if these cells are devoid of intracellular steroid receptors, as has been shown for neurons of the striatum, cortex, and cerebellum (Majewska, 1987b; O'Connor et al., 1988; Gee, 1988).

Since GABA is one of the most important inhibitory neurotransmitters in the central nervous system (Roberts, 1986) and GABAergic neurons and nerve terminals are ubiquitous in the brain (Mugnaini and Oertel, 1985), steroid hormones released from the gonads and adrenal glands may regulate numerous brain mechanisms by modulating GABAa receptor binding. That is, the activation of GABAa receptors is involved in the control of reproductive behavior (McGinnis et al., 1980; Fernandez-Guasti et al., 1985, 1986; Masco et al., 1986; Qureshi et al., 1988; McCarthy and Malik, 1988), the regulation of stress responses (Robel et al., 1986; Trullas et al., 1987; Schwartz et al., 1987;), and the modulation of pituitary gland activity (Masotto and Negro-Villar, 1986; McCann and Rettori, 1986; Wuttke et al., 1986; Fjalland et al.,1987). Steroid hormone action on the GABAa receptor also influences the sensitivity of the brain to seizures (Backstrom, 1976; Rociszewska et al., 1986; Schwartz-Giblin et al., 1989). In addition to its

role as an inhibitory neurotransmitter, GABA exerts trophic effects on the developing nervous system and plays a role in the morphogenesis of neurons (Michler-Stuke and Wolff, 1987; Hansen et al., 1987, 1988). Thus, steroid hormones may exert some of their differentiating effects on the developing brain by modifying the activity of GABAergic pathways. However, the molecular mechanisms, through the mediation of which gonadal and adrenal hormones modulate the GABAa receptor, are diverse and not well understood. The aim of the present review is to describe the complex organization of the GABAa receptor and to propose some mechanisms by which steroids may modulate its functions.

Heterogeneity of GABAa and Drug Binding Sites

Before discussing the effects that steroid hormones exert on the GABAa receptor, we need to describe the characteristics of the receptor complex and the diverse allosteric interactions between its drug binding sites. The GABAa receptor is a supramolecular complex consisting of a chloride ionophore and different binding sites for GABA, benzodiazepines (BZ), convulsants, like picrotoxin, barbiturates, anions, cations, and probably steroid hormones (Trifiletti et al., 1985; Squires, 1986; Enna and Karbon, 1986; Fischer and Olsen, 1986; Majewska 1987b; Gee et al., 1987; Gee 1988). This has been suggested by binding studies and confirmed by the observation that the solubilized and purified receptor protein still retains its different binding sites. Moreover, the allosteric interactions between these recognition sites can be preserved during purification (Sigel et al., 1983; Sigel and Barnard, 1984; Kirkness and Turner, 1986; King et al., 1987; Bristow and Martin, 1987; Stauber et al., 1987; Mamalaki et al., 1989; Dunn et al., 1989). By binding to the GABAa receptor, γ- aminobutyric acid (GABA) increases the neuronal membrane conductance to chloride ions, resulting in membrane hyperpolarization and reduced neuronal excitability. The GABA, BZ, and picrotoxin binding sites on the GABAa receptor all show multiple affinities.

The GABA Site

The GABA binding site can be selectively labeled by agonists like muscimol, isoguvacine and THIP (4,5,6,7-tetrahydoroisoxasolo (5,4) pyridine-3-ol). This site shows both high and low affinities for GABA, and its agonists with K_d values in the nanomolar or 10^{-7}–$10^{-6}M$ range, respectively, when assayed at 0°C. GABA binding sites from different brain regions, different mammalian species, and different ages all show this heterogeneity in affinity (Olsen et al., 1984). Even at higher temperatures (22 or 37°C), this heterogeneity in affinity for agonists is still observed (Yang and Olsen, 1987; Majewska, 1988). The low-affinity GABAa recognition site is an antagonist preferring site that can be selectively labeled by specific antagonists, like (+)bicuculline (Olsen and Snowman, 1983) or SR 95531 (Heaulme et al., 1987; McCabe et al., 1988). These different affinities can be explained by different affinity states of a single receptor protein. That is, both the low- and high-affinity forms of the GABAa site show similar drug specificity (Olsen et al., 1984) and are immunologically similar (De Blas et al., 1988), suggesting that they may represent different conformational states of a same protein. Moreover, membrane treatments like freezing and exposure to detergents, as well as the ionic concentration of the buffer, change the proportion of GABAa sites found in the different affinity states (Fischer and Olsen, 1986). These results can be interpreted as receptor subpopulation interconversion. In agreement with this hypothesis is the observation that pentobarbital increases the amount of high-affinity sites at the expense of low-affinity sites (Yang and Olsen, 1987). Thus, low- and high-affinity GABAa binding sites may correspond to different conformational states of a same receptor.

It is generally admitted that GABA exerts its physiological effects by acting on low-affinity sites. First, micromolar (μ*M*) concentrations of GABA or its analogs are necessary to activate the chloride channel (Nowak et al., 1982; Segal

and Barker, 1984), enhance BZ binding (Tallman et al., 1978, 1980), inhibit TBPS binding (Squires et al., 1983; Maksay and Ticku, 1985), and inhibit acetylcholine (Ach) release from rat striatal slices (Supavilai and Karobath, 1985). Second, abolishing high-affinity GABAa binding, without affecting the low-affinity component, by adding ammonium thiocyanate to the incubation medium does not impair the GABA-activated diazepam binding (Browner et al., 1981). Third, GABA inhibits evoked glutamate release from cultured cerebellar granule cells only when low-affinity GABAa sites are present on the cells. This inhibitory effect of GABA can be blocked by bicuculline (Meier et al., 1984). Taken together, these results suggest that the low-affinity GABAa binding sites are the physiologically relevant sites.

The Benzodiazepine Site

The BZ recognition site also appears to be heterogeneous. Binding experiments, performed with classical BZ agonists like diazepam, clonazepam, or flunitrazepam, reveal only a single population of BZ binding sites. However, a few ligands are able to discriminate between two subclasses of BZ binding sites. These compounds include triazolopyridazines (CL 218872) and β-carbolines, like propyl-β-carboline-3-carboxylate (PrCC) and β-carboline-3-carboxylate (DMCM) (Müller, 1987). These drugs are called "inverse agonists" because they do not simply antagonize the sedative effects of BZ, but also have convulsant properties by themselves (Lawrence et al., 1986). Triazolopyridazines and β-carbolines both inhibit BZ binding in a biphasic manner. Sites that bind these drugs with high affinity are called type 1 (BZ1), whereas type 2 (BZ2) benzodiazepine binding sites show little affinity for the inverse agonists (Müller, 1987).

There is evidence that the heterogeneity of BZ binding sites corresponds to different conformational states of a single receptor. First, BZ agonists do not discriminate between BZ1 and BZ2 sites. Thus, both binding sites are characterized by a similar drug specificity (Müller et al., 1987). Second, the characteristics of BZ binding are temperature dependent (Speth et al., 1979) and inverse agonists preferentially label the BZ1 subclass in vitro at 4°C, but not at 37°C (Gee et al., 1982, 1983; Gee and Yamamura, 1982; Sieghart et al., 1985). However, the triazolopyridazine CL 218872 still discriminates between low- and high-affinity sites at 37°C (Gee et al., 1984). Another observation also suggests that BZ1 and BZ2 sites may correspond to different conformations of a single site. Thus, the inhibition constants (K_i) and the maximal inhibition values for flunitrazepam displacement of the β-carboline, PCC, or flunitrazepam binding are similar (Ehlert et al., 1983). Thus, the BZ recognition site heterogeneity may be related to different conformational states of the same receptor. However, the molecular basis of BZ receptor subtypes is not well understood, and it is actually unknown whether BZ1 and BZ2 sites are interconcertible.

The Convulsant Site

In addition to picrotoxin, there are two other specific ligands for the convulsant binding site: dihydropicrotoxin and *t*-butylbicyclophosphorothionate (TBPS) (Lawrence and Casida, 1983). Displacement (Squires et al., 1983) and equilibrium binding studies, which have been carried out over a wide range of TBPS concentrations (Tehrani et al., 1985), show that TBPS binding is also heterogeneous and characterized by low and high affinities. In the presence of GABA, clonazepam inhibits TBPS binding (Gee et al., 1986; Lawrence et al., 1986).

Conclusions

GABA, BZ, and picrotoxin binding sites, located on the GABAa receptor, all show different affinities for their respective agonists or antagonists. These different affinities may correspond to different conformations of the receptor complex. That is, experimental results suggest that the high and low affinities of the GABA and the convulsant sites are interconvertible. Whether this is also the case for BZ2 and BZ1 sites still has

to be determined. The conformational changes of the GABAa receptor may be regulated by allosteric interactions between the different drug binding sites, as described below. These interactions may be described in terms of either positive or negative heterotropic cooperativity (Ehlert, 1986).

Allosteric Interactions Between the Different Drug Binding Sites

The different drug recognition sites and the chloride ion channel of the GABAa receptor complex interact in an allosteric manner. These complex and multiple interactions are described in detail in the following sections and summarized in Table 1.

GABA Modulation of the Benzodiazepine Site

Diazepam binding to the BZ site is enhanced by GABA and muscimol. This enhancement is antagonized by (+)bicuculline (Tallman et al., 1978; Karobath et al., 1979; Krogsgaard-Larsen et al., 1986). In cortex, cerebellum, striatum, and hippocampus, GABA increases the affinity of the BZ binding sites for flunitrazepam without altering their binding capacity (Wastek et al., 1978; Tallman et al., 1978). Two studies suggest that GABA specifically increases agonist binding to the BZ2 site. Thus, 1 μM GABA increases the binding of flunitrazepam (agonist) by 20%, and 10 μM GABA increase the binding of this ligand by almost 50%. By contrast, 1 μM GABA increases the binding of the inverse agonist PrCC only by 1%, and higher concentrations of GABA do not cause a further increase in PrCC binding (Fehske et al., 1982). Similarily, in both hippocampus and cortex, the binding of inverse agonists is not regulated by GABA (Fehske et al., 1982; Medina et al., 1989). Thus, GABA may stabilize a conformation of the BZ site, which has a high affinity for agonists. However, it is also possible that GABA fails to regulate the binding of β-carbolines, because these drugs are inverse agonists. That is, inverse benzodiazepine agonists can be considered as allosteric GABAa antagonists, and their binding may not be modulated like the binding of benzodiazepine agonists.

Benzodiazepine Modulation of the GABA Site

Reciprocically, BZs increase the binding of GABA to its recognition site. Kinetic studies show that diazepam increases the affinity of the low-affinity component of GABA binding without altering its binding capacity. High-affinity sites for GABA are unaltered by BZs (Skerritt et al., 1982). As a result, BZs potentiate the physiological effects of GABA. However, they are ineffective on the chloride ionophore by themselves. Thus, BZs do not alter the spontaneous firing rate of serotonergic dorsal raphe nucleus neurons, but potentiate the inhibitory response produced by GABA (Gallager, 1978). Similarily, BZs potentiate the inhibitory effects of GABA on acetylcholine release (Supavilai and Karobath, 1985). Thus, there exist mutual interactions between GABA and BZ sites: GABA increases the affinity of BZ sites for their agonists, whereas BZs increase the affinity of low-affinity GABAa sites for GABA and its agonists. These interactions between GABA and BZ binding sites are chloride ion dependent (Costa et al., 1979).

Electrophysiological studies have shown that GABA activates two-state (open-closed) chloride ion channels by increasing their conductance (Segal and Barker, 1984). Diazepam potentiates the increase in chloride ion conductance produced by GABA by increasing the frequency of channel opening. By itself, diazepam has no effect on chloride channel opening (Study and Barker, 1981). The dose-response curve for the chloride ion conductance increasing effect of GABA is shifted to the left by BZs without change in its maximum (Haefely and Polc, 1986). This is in agreement with the observation that BZs increase the affinity of the GABA binding site without affecting the number of functional receptors.

Table 1
Allosteric Interactions Between the Different Drug Binding Sites of the GABAa Receptor Complex[a]

Drug	Target	Effects	References
GABA agonists	BZ2 site	↑ affinity for agonists	Tallman et al., 1978 Wastek et al., 1978 Karobath et al., 1979 Krogsgaard-Larsen et al., 1986
	BZ1 site	no effect on inverse agonist binding	Fehske et al., 1982 Medina et al., 1989
	chloride channel	↑ Cl^- ion conductance	Segal and Barker, 1984
	convulsant site	↓ binding of convulsants like TBPS	Squires et al., 1983
BZ agonists	GABAa site	↑affinity for agonists	Skeritt et al., 1982
		↑ physiological effects of GABA	Gallager, 1978 Supavilai and Karobath, 1985
	chloride channel	↑ frequency of channel opening	Study and Barker, 1981
	convulsant site	↓ binding of convulsants	Squires et al., 1983 Lawrence et al., 1986 Gee et al., 1986
BZ inverse agonists	convulsant site	↑ the number of high-affinity sites	Lawrence et al., 1986
Anions	GABAa site	↑ binding to low-affinity sites ↓ binding to high-affinity sites	Enna and Snyder, 1977
	GABA/BZ sites	required for their interactions	Costa et al., 1979
	convulsant site	required for convulsant binding	Bowery et al., 1976 Willow and Johnston, 1980 Olsen and Snowman, 1982 Leeb-Lundberg and Olsen, 1983 Squires et al., 1983 Maksay and Ticku, 1985
Barbiturates	GABAa site	↑ the number of high-affinity sites	Willow and Johnston, 1980 Olsen and Snowman, 1982 Yang and Olsen, 1987
		↓ the binding of antagonists	Wong et al., 1984 McCabe et al., 1988
	BZ site	↑ affinity for agonists	Leeb-Lundberg et al., 1980 Majewska et al., 1986 Fischer and Olsen, 1986
		↓ binding of inverse agonists	Wong et al., 1984
	chloride channel	↑ the average open-time	Study and Barker, 1981
	convulsant site	↓ TBPS binding	Squires et al., 1983
Pyrazolopyridines	GABAa site	↑ affinity for agonists	Leeb-Lundberg et al., 1981 Leeb-Lundberg and Olsen, 1983
	BZ site	↑ affinity for agonists	Leeb-Lundberg et al., 1981 Leeb-Lundberg and Olsen, 1983
	convulsant site	↓ binding of TBPS and picrotoxin	Leeb-Lundberg et al., 1981 Squires et al., 1983
		↑ the dissociation rate for TBPS	Maksay and Ticku, 1985
Convulsants	GABAa site	block the affects of barbiturates	Willow and Johnston, 1980 Olsen and Snowman, 1982
	BZ site	block the effects of barbiturates	Leeb-Lundberg and Olsen, 1983
	chloride channel	block barbiturate effects	Takeuchi and Takeuchi, 1969

[a]BZ benzodiazepines; TBPS = *t*-butyl-bicyclophosphorothionate.

Barbiturate Modulation of the GABAa Receptor

The barbiturate binding site interacts with both GABA and BZ and convulsant recognition sites. In the presence of chloride ions, barbiturates enhance GABA binding. This increase in GABA binding has been shown to involve an increase in the number of both low- and high-affinity sites (Olsen and Snowman, 1982), high-affinity sites without affecting low-affinity sites (Willow and Johnston, 1980), or selectively, high-affinity sites at the expense of low-affinity sites (Yang and Olsen, 1987). Barbiturates also inhibit the binding of GABAa antagonists, like (+)bicuculline (Wong et al., 1984) or SR 95531 (McCabe et al., 1988), that selectively label the low-affinity GABAa binding site in the presence of anions (Möhler and Okada, 1978; Olsen and Snowman, 1983; Heaulme et al., 1987; McCabe et al., 1988). These results suggest that barbiturates may change the conformation of low-affinity GABAa binding sites (antagonist preferring sites) to a higher affinity state (agonist preferring state). Barbiturates also increase, in an anion dependent and reversible manner, the affinity of BZ binding sites without affecting their number (Leeb-Lundberg et al., 1980; Majewska et al., 1986; Fischer and Olsen, 1986). In fact, there is a good correlation between the biological activity of different barbiturates and their ability to enhance BZ binding (Leeb-Lundberg et al., 1980). By contrast, the binding of β-carbolines is inhibited by barbiturates (Wong et al., 1984). Thus, barbiturates increase the affinity of the BZ sites for agonists and reduce their affinity for inverse agonists. These interactions between the GABAa and BZ sites can be best explained in terms of positive or negative heterotropic cooperativity, respectively (Ehlert, 1986).

Electrophysiological studies have shown that barbiturates, like pentobarbital, enhance the GABA-stimulated chloride ion conductance in neurons. The shift of the GABA dose-response curve for chloride ion conductance suggests that pentobarbital increases the affinity of the GABA recognition site for its ligand (Barker and Ransom, 1978). This observation is in agreement with results obtained from binding studies that we described earlier. In contrast to BZs, which increase the frequency of channel opening, pentobarbital potentiates the increase in chloride ion conductance produced by GABA by increasing the average open-channel lifetime (Study and Barker, 1981).

Convulsant Modulation of the GABAa Receptor

Picrotoxin blocks the effects of pentobarbital on GABA-activated chloride ion conductance (Takeuchi and Takeuchi, 1969). Picrotoxin also reverses the effects of barbiturates on GABA (Willow and Johnston, 1980; Olsen and Snowman, 1982) and BZ binding (Leeb-Lundberg et al., 1981; Leeb-Lundberg and Olsen, 1983). These effects of picrotoxin, again, are chloride ion dependent. The observation that picrotoxin blocks the enhancement of BZ binding by pentobarbital, but not the enhancement produced by GABA (Leeb-Lundberg et al., 1981), shows that the picrotoxin and GABA recognition sites are distinct. This has been confirmed by two recent studies (Ramanjaneyulu and Ticku, 1984; Trifiletti et al., 1985). However, picrotoxin and TBPS not only inhibit the increase of GABA and BZ binding by barbiturates, but the binding of these convulsants to the picrotoxin site, in turn, is inhibited by BZs, GABA, and barbiturates (Squires et al., 1983). GABA inhibits TBPS binding by accelerating its dissociation rate (Maksay and Ticku, 1985) and reducing the effectiveness of Eccles anions (Cl^-, Br^-, I^-, and SCN^-) in increasing TBPS binding (Squires et al., 1983). In the presence of GABA, clonazepam inhibits TBPS binding (Gee et al., 1986; Lawrence et al., 1986). By contrast, β-carbolines increase the number of high-affinity TBPS binding sites. These observations can be interpreted as an interconversion of TBPS binding sites by BZ agonists or inverse agonists and may reflect either positive or negative heterotropic cooperativity, depending on whether an agonist or an inverse agonist binds to the BZ site (*see* Ehlert, 1986). According to the

principle of heterotropic cooperativity, high-affinity TBPS sites may be associated with BZ1 sites, whereas low-affinity TBPS sites may be associated with BZ2 sites.

Pyrazolopyridine Modulation of the GABAa Receptor

The anxiolytic pyrazolopyridines, etazolate (SQ 20009) and cartazolate (SQ 65396), inhibit dihydropicrotoxin binding (Leeb-Lundberg et al., 1981) and TBPS binding (Squires et al., 1983) by accelerating their dissociation (Maksay and Ticku, 1985). Both etazolate and cartazolate also enhance the binding of diazepam to the BZ recognition site by increasing its affinity, without affecting its binding capacity. They also increase the affinity of the GABA binding site for muscimol (Leeb-Lundberg et al., 1981; Leeb-Lundberg and Olsen, 1983). Thus, pyrazolopyridines exert barbiturate-like effects on BZ and GABA binding.

The Role of Anions

Anions, like chloride, play an important role in regulating the complex allosteric interactions between the different drug binding sites. Thus, the positive interactions between the GABA and BZ binding sites are chloride-ion dependent (Costa et al., 1979). Anions, like chloride and SCN^-, also increase the binding of GABA to the low-affinity GABAa recognition site and reduce the binding of GABA to its high-affinity site (Enna and Snyder, 1977). Barbiturates enhance GABA and BZ binding only if chloride ions (or other anions like I^-, Br^-, or SCN^-) are present (Leeb-Lundberg et al., 1980; Olsen and Snowman, 1982). Similarily, the inhibitory effects that picrotoxin exerts on barbiturate-enhanced GABA binding are strictly chloride ion dependent (Leeb-Lundberg et al., 1981). The inhibitory effects of TBPS and its related convulsants on the GABA-activated chloride ion conductance are also dependent on the presence of anions (Bowery et al., 1976). Thus, it is not surprising that the binding of convulsants to the picrotoxin/TBPS binding site also requires the presence of anions (Squires et al., 1983). That is, the rate of TBPS dissociation is accelerated by the removal of chloride ions (Maksay and Ticku, 1985).

Conclusions

The complex interactions between the different binding sites of the GABAa receptor are summarized in Table 1 and can be outlined as follows. By binding to their respective sites, BZ agonists and barbiturates increase high-affinity GABA binding and potentiate its physiological actions. However, whereas BZs increase the frequency of chloride channel opening in the presence of GABA, barbiturates increase the open-channel lifetime. By contrast, the binding of convulsants, like picrotoxin and TBPS, is inhibited by both barbiturates and BZs, but is increased by BZ inverse agonists. These convulsants, in turn, block the effects of barbiturates on the GABA site and the chloride channel. The interactions between the GABA and BZ sites are reciprocal: GABA increases the affinity of BZ agonist binding, and BZs increase the affinity of low-affinity GABA sites. Finally, pyrazolopyridines, like etazolate and cartazolate, exert barbiturate-like effects on GABA and BZ binding. Most of the allosteric interactions between different binding sites and the chloride ion channel are anion dependent.

The "Three-State Model" for the GABAa Receptor

There is strong evidence that the different affinities of GABAa, BZ, and TBPS binding sites correspond to different affinity states of a single receptor protein, rather than to distinct receptor subtypes. This led several authors to propose allosteric models for the GABAa receptor that are similar to the one proposed by Changeux and collaborators for the nicotinic acetylcholine receptor (Changeux et al., 1984; Changeux and Revah, 1987). According to Fischer and Olsen

(1986), the GABAa binding site is supposed to exist in three different conformational states: a resting state (super-low affinity; chloride channel inactive), an activated state (low affinity, channel active), and a desensitized state (high affinity, channel inactive). When GABA binds to the resting state, it is converted rapidly to an active state. While remaining bound to the receptor, GABA converts the active state to a desensitized, high-affinity state. The different affinity states of the GABAa recognition site are all supposed to be present at equilibrium during in vitro binding assays, and this equilibrium can be altered by anions (stabilizing the low-affinity form) or barbiturates (stabilizing the high-affinity form) (Fischer and Olsen, 1986).

Ehlert and collaborators also presented an allosteric model for the BZ acceptor site (Ehlert et al., 1983). Essentially, this model is based on the observation that GABA increases the binding of BZ to BZ2 sites, but not to BZ1 sites. According to this model, the macromolecular GABAa receptor complex may exist in two states: an "inactive state," characterized by a closed chloride ion channel, and an "active state" corresponding to an open chloride ion channel. In the absence of GABA, the "inactive state" is preferred and most of the BZ recognition sites correspond to BZ1 sites showing a high affinity for inverse agonists. When GABA binds to the complex, the "active state" is preferred, and the BZ binding sites are stabilized in the BZ2 conformation, which has a reduced affinity for inverse agonists and is functionally coupled to the GABAa recognition site. However, it still has to be shown that BZ2 and BZ1 sites correspond to different interconvertible conformations of a single receptor. Moreover, the possibility that BZ1 sites may be associated with a closed conformation of the chloride channel has not been explored experimentally.

Fischer and Olsen (1986) then proposed a model in which the multiple allosteric states of the BZ binding sites fit with the three-state model for the GABAa recognition site (Fig. 1). According to their model, the desensitized, inactive configuration of the GABAa receptor complex is characterized by high-affinity binding sites for GABA and high-affinity sites for inverse BZ agonists, like β-carbolines (BZ1 sites). By contrast, the active configuration of the GABAa receptor is characterized by low-affinity sites for GABA (preferentially labeled by antagonists like (+)bicuculline) and BZ binding sites showing low-affinity for β-carbolines and high-affinity for BZ agonists (BZ2 sites). Such a model would explain the observation that GABA enhances BZ binding to BZ2 sites by binding to low-affinity sites (Tallman et al., 1978; Karobath et al., 1979), but does not affect BZ binding to BZ1 receptors while binding to inactive high-affinity sites (Gee et al., 1983). It is also not difficult to integrate the different affinity states of the TBPS binding sites into this model. High-affinity TBPS binding sites, which may be associated with a closed ionophore, would be characteristic of the inactive configuration of the GABAa receptor showing high-affinity for GABA and β-carbolines. Low-affinity TBPS binding sites would correspond to the active configuration of the receptor complex (low affinity for GABA and β-carbolines and high-affinity for (+)bicuculline). This model would explain the observations that β-carbolines stabilize high-affinity TBPS binding sites, whereas GABA (Gee, 1988) and BZ agonists stabilize the low-affinity TBPS binding (Gee et al., 1986).

A "Five-State Model" for the GABAa Receptor

The "Three-State Model," described above, proposes that the GABAa receptor may adopt as many as three interconvertible conformations. According to this model, low-affinity GABA binding sites may be associated with the active conformation of the receptor complex. On the other hand, high-affinity GABA binding sites would correspond to the inactivated receptor. However, this model does not explain the effects of barbiturates, pyrazolopyridines, and benzodiazepines. Describing the modulation of the GABAa receptor by these drugs, in terms of

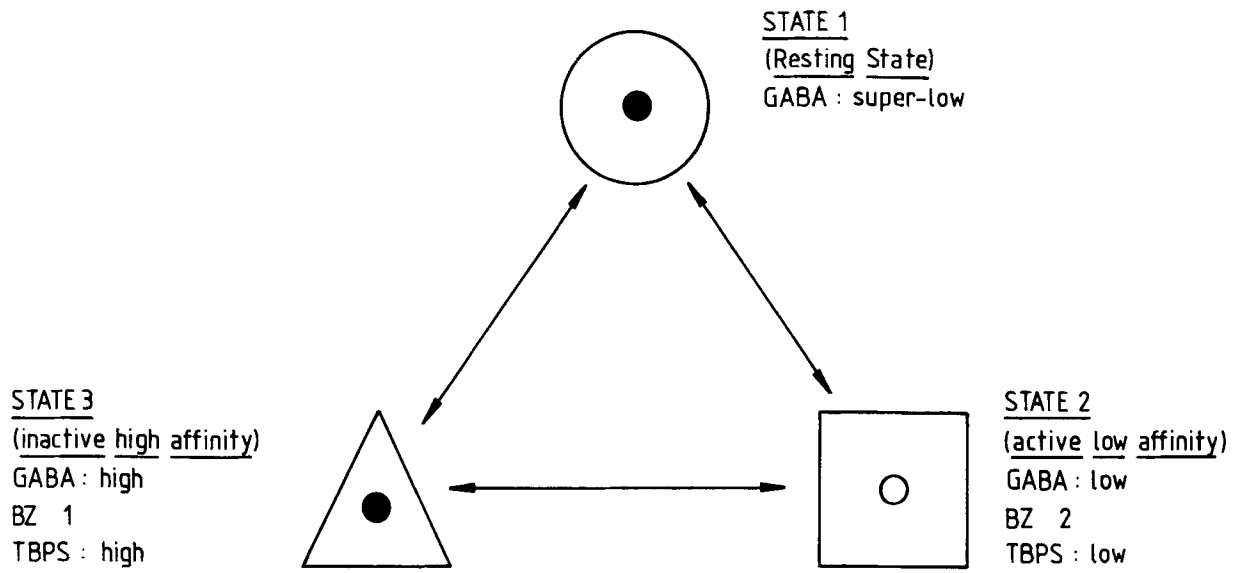

Fig. 1. The "Three-State Model" for the GABAa receptor, as proposed by Fischer and Olsen (1986). State 1 is the resting state, which is inactive and has a very low affinity for GABA and its agonists. When GABA binds to the resting state, it is converted to a low-affinity state that is active (State 2). State 3 is the desensitized state, which shows a high affinity for both GABA and convulsants, like TBPS or picrotoxin.

conformational changes, requires postulating the existence of two additional conformations that show a high affinity for GABA, but are also functional. That is, barbiturates, pyrazolopyridines, and benzodiazepines stabilize receptor conformations where high-affinity GABA sites are functionally coupled to the chloride ionophore. These high-affinity conformations differ from the inactive high-affinity state of the receptor (= state 3 of the "Three-State Model") by their low affinity for convulsants and BZ inverse agonists. That barbiturates and pyrazolopyridines may stabilize a different high-affinity conformation of the GABAa receptor than BZs is suggested by electrophysiological observations. This has led us to propose a "Five-State Model" for the GABAa receptor. Our model predicts that, in addition to the "resting superlow-affinity state," the "active low-affinity state," and the "inactive high- affinity state" proposed by the "Three-State Model," two "active high-affinity conformations" may exist (Fig. 2). In agreement with such a hypothesis of multiple active conformations of the GABAa receptor, is the observation that GABA stimulated chloride-ion exchange can be attributed to two different receptor conformations (Cash and Subbarao, 1988).

Some of the evidence for such additional active conformations is presented below. Barbiturates, which change the conformation of the low-affinity GABAa binding sites to a higher affinity state, would, according to the three-state model, shift the active low-affinity configuration of the receptor to the inactive high-affinity configuration, characterized by high-affinity binding sites for β-carbolines and TBPS and a closed chloride ion channel. However, this is contradicted by several observations. First, barbiturates potentiate the effects that GABA exerts on chloride ion conductance. Second, barbiturates enhance BZ agonist binding and decrease the affinity for β-carbolines in a reversible manner (Wong et al., 1984). Third, barbiturates decrease the affinity of TBPS binding sites (Squires et al., 1983). Thus, barbiturates potentiate the physiological effects of GABA by increasing the affinity of the receptor for GABA and BZ agonists and decreasing its affinity for convulsants like TBPS. Similarily,

pyrazolopyridines, like etazolate and cartazolate, exert anxiolytic effects by enhancing both GABA and BZ binding and inhibiting TBPS binding. The easiest way to explain these effects of barbiturates and pyrazolopyridines on the GABAa receptor by a conformational change is to postulate that these drugs may stabilize a fourth conformation of the GABAa receptor (= State 4, *see* Fig. 2) that is in equilibrium with the previously described three affinity states. In this conformation, high-affinity GABAa recognition sites would be functionally coupled to the chloride ion channel and the BZ binding site, whereas the picrotoxin/TBPS site would show "super-low" affinity for its ligands. The fact that high-affinity GABAa binding sites can be functionally coupled to BZ binding sites has been shown by the observation that pretreatment of membranes with silver ions or Triton X-100 leads to the sole appearance of high-affinity muscimol binding, which is still able to increase flunitrazepam binding. As predicted by the five-state model, TBPS binding sites are not present longer in these treated membranes (Supavilai and Karobath, 1984). However, the latter observation may result from a denaturation of the TBPS binding site by the detergent.

There is also reason to postulate another state of the receptor complex. As described above, the binding of GABA to its recognition site increases the affinity of the BZ binding site, and reciprocally, the binding of BZs enhances the affinity of the low-affinity GABA binding site. Simultaneous occupation of both GABAa and BZ sites not only increases the affinity of the receptor for GABA and BZ, but also decreases its affinity for TBPS (Squires et al., 1983). Thus, the conformational changes caused by GABA and BZ agonists appear to be similar to those caused by barbiturates. However, there are differences between BZs and barbiturates, with respect to their effects on channel opening. Whereas barbiturates increase the open-channel lifetime and decrease the frequency of channel opening caused by GABA, BZ agonists increase the frequency of channel opening in the presence of GABA (Study and Barker, 1981; Mienville and Vicini, 1989). One could speculate that BZs may induce a different allosteric change in the receptor oligomer than barbiturates (Schwartz, 1988). Thus, binding of GABA and BZ to their respective sites may stabilize a fifth conformational state that differs from the conformation favored by barbiturates, with respect to channel kinetics (= State 5, *see* Fig. 2).

GABAa Receptor Subunits and Subtypes

Although the above proposed drug-dependent conformational changes of the GABAa receptor account for numerous experimental data when homogenized brain tissue is used, there are some observations that cannot be explained by the allosteric model. That is, agonist and drug binding to the GABAa receptor appears to be heterogeneous. Autoradiographic studies have shown that the neuroanatomical distribution of high- and low-affinity GABA binding sites does not always correspond (McCabe and Wamsley, 1986). This indicates that different GABAa receptor complexes may exist within different brain regions. Similarily, the heterogeneity that affects the BZ recognition site can also be interpreted in terms of distinct receptor populations. Thus, the relative proportion of BZ1 and BZ2 sites varies between brain regions (Braestrup and Nielsen, 1981; Fehske et al., 1982), and receptors characterized by BZ1 and BZ2 binding sites, respectively, correspond to distinct peptides on SDS gels (Sieghart et al., 1983). Moreover, BZ1 and BZ2 sites also differ on the basis of their solubilization properties and their sensitivity to ions (Lo et al., 1982).

Several mechanisms may account for the observed heterogeneity of the GABAa receptor complex. Thus, the subunit composition of the GABAa receptor may vary between different brain regions or different developmental stages. This would explain why the allosteric modulation of the GABAa receptor differs between brain areas (*see below*) and changes with age (Belhage

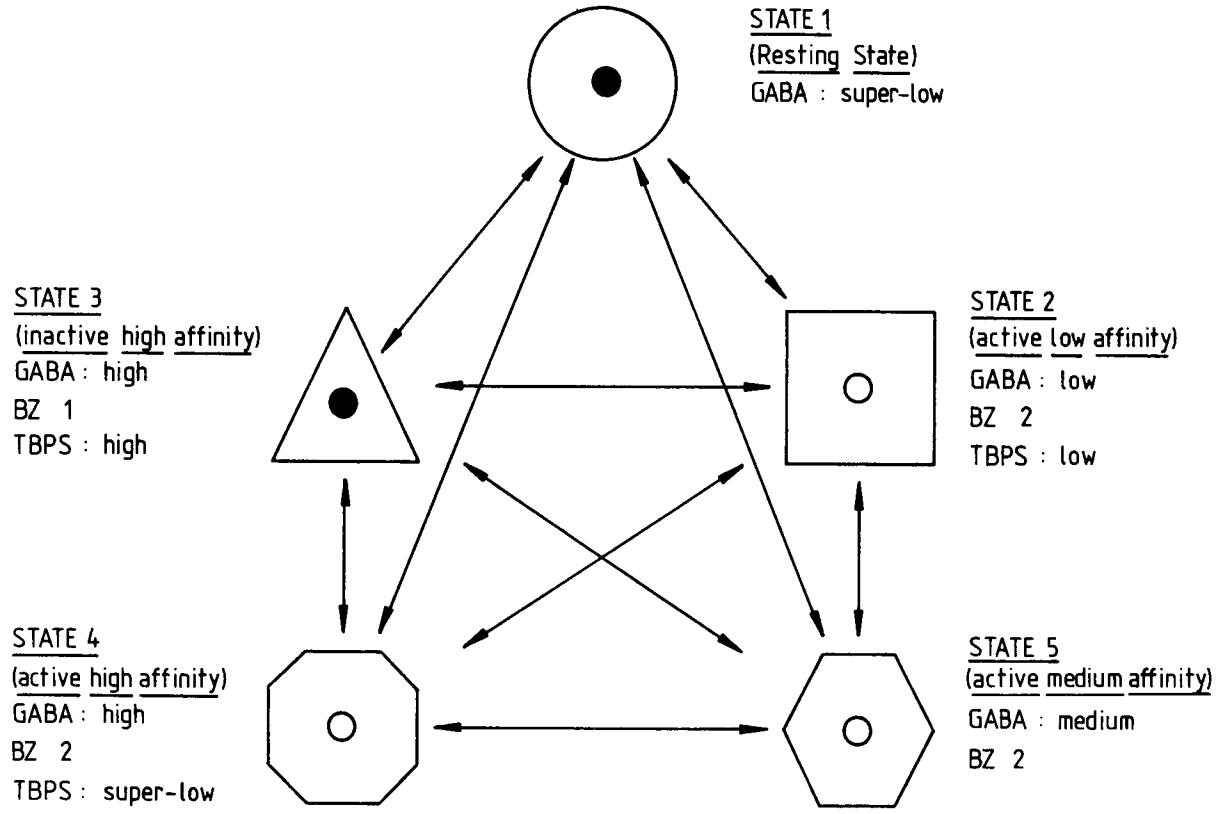

Fig. 2. The "Five-State Model" for the GABAa receptor. The effects that barbiturates and benzodiazepines exert on the receptor complex suggest the existence of two additional conformations, compared with the "Three-State Model" (*see* Fig. 1). State 4 is an active conformation of the receptor, which shows a high affinity for both GABA and BZ agonists and a very low affinity for convulsants. This state is stabilized by barbiturates and, possibly, by 3α-steroids. State 5 results from the activation of State 2 by the simultaneous binding of GABA and BZ to the receptor.

et al., 1988). That is, the GABAa receptor complex purified to homogeneity from bovine cerebral cortex, appears to be a heterotetramer of homologous alpha (M_r = 53 kD) and beta (M_r = 56 kD) subunits. The subunit structure has been shown to be α2β2 (Mamalaki et al., 1987; Casalotti et al., 1986). Recently, a γ- subunit has been isolated that shares approximately 40% sequence identity with the α and β-subunits and is important for the response of the receptor to BZs (Pritchett et al., 1989). Additional GABAa receptor subunits may exist (Pritchett et al., 1989). The α, β, and γ subunits from human, bovine, or rat brain have been sequenced, cloned, and expressed in *Xenopus* oocytes (Schofield et al., 1987; Lolait et al., 1989; Pritchett et al., 1989). Photoaffinity labeling experiments have suggested that the BZ binding sites may be located on α-subunits, whereas the binding sites for GABA agonists may reside on β-subunits (Deng et al., 1986; Kirkness and Turner, 1986; Mamalaki et al., 1987; Casalotti et al., 1986). However, recent evidence suggests that both subunits carry binding sites for GABA and BZs (Bureau and Olsen, 1988).

The fact that a different subunit composition may account for the heterogeneity of a macromolecular receptor complex has already been shown for the nicotinic acetylcholine receptor (AchR). This receptor is composed of five sub-

units (α2, β, γ, and δ) that surround a cation-sensitive ion channel (Changeux and Revah, 1987). In early development, the AchR is assembled from α, β, γ, and δ subunits, whereas in the adult receptor, the γ-subunit is replaced by a σ-subunit (Mishina et al., 1986; Kidokoro, 1988). As a consequence, the AchR types present in adult or fetal muscle differ in conductance and gating properties. Such changes in subunit composition during development have also been reported for the GABAa receptor. GABAa receptor complexes, purified from neonatal rat cortex, contain predominantly β-subunit-like proteins, whereas receptors from adult cortex contain nearly equal amounts of α- and β-subunit-like proteins (Sato and Neale, 1989). Differences in subunit composition for the GABAa receptor seem also to exist between different brain regions. Whereas adult cerebellar receptors contain predominantly type 1 benzodiazepine binding sites, cortical receptors contain a mixture of both type 1 and type 2 sites (Sato and Neale, 1989). The latter observation fits well with a recent study showing that, in the cerebellum, both flunitrazepam and *n*-butyl-beta-carboline-3-carboxylate label the same number of sites, whereas in the cortex, flunitrazepam binding is twice the binding of *n*-butyl-beta-carboline-3-carboxylate (Medina et al., 1989).

Thus, a different subunit composition may account for the different GABAa receptor complexes that are present in different brain regions. That is, recent *in situ* hybridization results indicate that different subunits are expressed, to varying degrees, in different brain regions (Séquier et al., 1988). Moreover, cDNAs for three different α-subunits have been isolated, thus confirming the heterogeneous nature of the GABAa receptor complex. These three subunits differ in both size and sequence. In receptor reconstitution experiments, the different α-subunits confer different sensitivities to the receptor (Levitan et al., 1988). A recent study shows that the different α-subunit mRNAs (α1, α2, and α3) are differentially expressed within different brain regions (Wisden et al., 1989). That is, α2- and α3-transcripts are especially abundant in the striatum, whereas the α1-transcript is characteristic of the inferior colliculus, the olfactory bulb, and the substantia nigra, where BZls are numerous (Wisden et al., 1989). However, in addition to different subunit compositions, the presence of different regulatory factors within distinct brain region may also contribute to the neuroanatomical heterogeneity of the GABAa receptor. That is, the different subunits are targets for glycosylation (Sweetman and Tallman, 1986; Mamalaki et al., 1987) and phosphorylation processes (Kirkness et al., 1989), and there is experimental evidence suggesting that the GABAa receptor can be regulated by cAMP-dependent phosphorylation (Heuschneider and Schwartz, 1989).

Conclusions

The GABAa receptor complex is composed of at least three subunits, which are called α, β, and γ, respectively. Recent cloning experiments suggest that receptor subunits other than α, β, and γ may exist. Different GABAa receptor populations, which are associated with different brain regions or different developmental stages, may be composed of different subunits. Despite the widespread distribution of GABAa receptors in the brain (McCabe and Wamsley, 1986; Bowery et al., 1987), the observation that GABAergic agents exert subtle and specific effects on brain functions also suggests that there may be distinct subgroups of GABAa receptors that may be selectively manipulated for therapeutic gain (Enna and Karbon, 1986). However, the presence of different GABAa receptor subtypes *per se* is not sufficient to explain most of the electrophysiological and pharmacological observations. The different drug binding sites of the GABAa receptor, which are localized on the different subunits, interact with each other in an allosteric manner, and their activation causes conformational changes of the receptor protein that determine its functional properties. These interactions between binding sites, however, may depend, in turn, on the subunit composition of the receptor and, thus, may differ between

receptor populations. As a consequence, both subunit expression and capacity for neuromodulation may change during development or differ between brain regions.

The five-state model we proposed for the GABAa receptor implies that all the components necessary to form all states of the receptor are present. This is certainly not the case for all GABAa receptor populations. Thus, all the allosteric interactions we described in the five-state model may only be observed as an average property in homogenized tissue and within those distinct brain regions where the GABAa receptor is modulated by benzodiazepines, barbiturates, and steroid hormones. In fact, some GABAa receptor subtypes may be insensitive to some drugs or hormones because they lack the corresponding binding sites. Moreover, the number of sites residing on the receptor may determine its sensitivity to a given drug or hormone and the extent of the induced conformational change.

Although the possibility of conformational changes may be restricted for some GABAa receptor subtypes by the amount of associated subunits, the potential states are likely to correspond to the ones proposed by the five-state model. This means that, even if a GABAa receptor subtype lacks one type of binding site, ligand binding to sites that are present on the receptor can be expected to cause the conformational changes that are predicted by the five-state model. In other words, even if a GABAa receptor subpopulation lacks, for example, benzodiazepine binding sites, barbiturates should still stabilize an active high-affinity conformation of the GABAa receptor, provided the barbiturate site and chloride channel are present.

It follows that different GABAa receptor populations may differ in their sensitivity to gonadal and adrenal steroids by at least two mechanisms. First, by acting on gene expression, steroids may regulate the synthesis of different subunits in a specific manner. Second, because steroid hormones also have been shown to modulate the GABAa receptor by acting directly on the receptor complex, they may affect its activity by changing its conformation. The next two paragraphs will describe the effects that steroids exert on the GABAa receptor either in vivo or in vitro.

In Vivo Effects of Steroid Hormones

Having described the organization of the GABAa receptor complex, we can now explore the effects of steroid hormones. Both acute and chronic steroid treatments have been shown to affect the central GABAa receptor. Thus, short-term treatment of ovariectomized female rats with estradiol benzoate (EB) decreases high-affinity muscimol binding within several hormone sensitive brain regions, like the ventromedial nuclei (VMN) of the hypothalamus and the midbrain central gray (MCG) (O'Connor et al., 1988; Schumacher et al., 1989b), but increases muscimol binding within the CA1 region of the dorsal hippocampus (Schumacher et al., 1989a) (Fig. 3). When administered alone to ovariectomized females, progesterone (P) has no effect. However, in estrogen-primed females, P increases muscimol binding, in both VMN and MCG, to levels seen in control animals (Schumacher et al., 1989b). These observations raise two fundamental questions. First, why do steroid effects on GABAa receptor binding differ between brain regions? Second, through the mediation of what mechanisms do different steroid hormones exert different effects on the receptor complex? Thus, it is surprising that estrogens and progestagens exert opposite effects on high-affinity muscimol binding since the sequential actions of both hormones facilitate reproductive functions, like mating behavior and the release of gonadotropins (Boling and Blandau, 1939; Rubin and Barfield, 1983; McEwen et al., 1987). This observation suggests that E and P may affect muscimol binding by different mechanisms. Thus, estrogens may

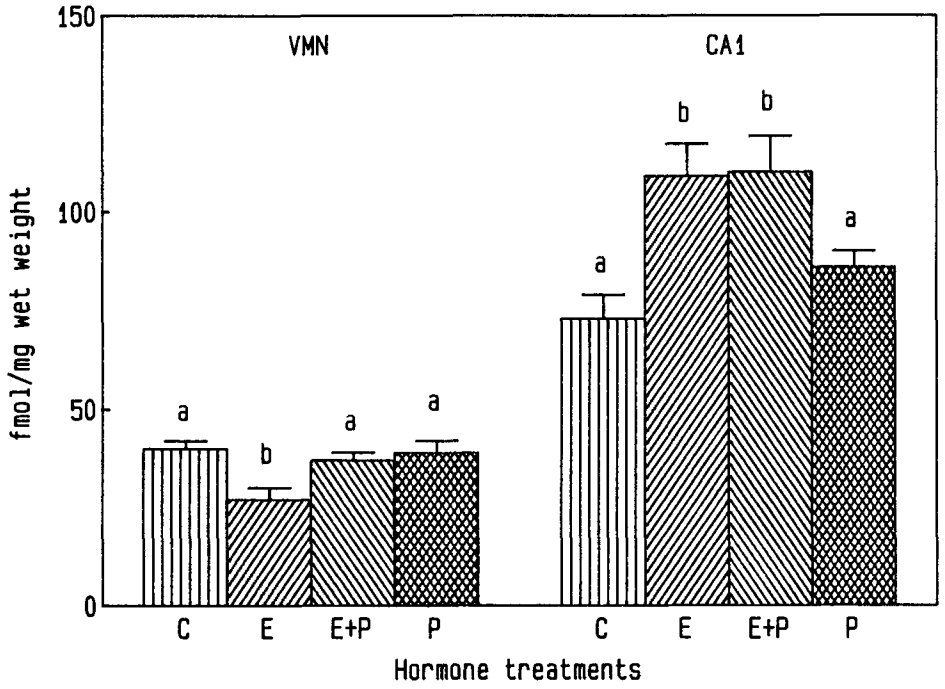

Fig. 3. Specific high-affinity ^{3}H-muscimol binding in the ventromedial nuclei of the hypothalamus (VMN) and the CA1 region of the dorsal hippocampus. Ovariectomized and adrenalectomized female rats (C females) were injected with estradiol benzoate (EB) (2 × 10 g, E females), progesterone (0.5 mg, P females), or successively with EB + P (E + P females). The binding of ^{3}H-muscimol was quantified by the method of in vitro receptor autoradiography. Different letters above the columns denote statistical differences at least at the 0.05 level within each area (modified from Schumacher et al., 1989a,b).

modulate the number of GABAa binding sites by regulating the expression of receptor genes, whereas progestagens, which affect muscimol binding within 4 h, may act directly on the receptor by causing conformational changes. That is, in vitro studies suggest that 5α-reduced metabolites of P may shift the conformation of the GABAa receptor from a low- to a high-affinity state (*see below*). According to our five-state model, such an effect of P may correspond either to an activation of the receptor (stabilization of an active high-affinity conformation) or its inactivation (stabilization of an inactive high-affinity conformation). It is unknown whether E and P exert these effects by acting directly on the receptor or through the mediation of their metabolites. Thus, catecholestrogens have been shown to modulate GABAa receptor binding (Etchegoyen et al., 1986), and reduced metabolites of P exert direct effects on the GABAa receptor (*see below*).

When reviewing the literature of hormone effects on the GABAa receptor, it appears that numerous apparently contradictory results have been reported (Table 2). By using the method of in vitro receptor autoradiography, estrogens have been shown, in one study, to decrease muscimol binding only within estrogen-concentrating areas of the rat brain (Schumacher et al., 1989b), but a similar hormone treatment has also been reported to decrease muscimol binding in brain regions, like the frontal cortex, that contain no estrogen concentrating cells (O'Connor et al., 1988). Such discrepancies may result from

Table 2
Regulation of Agonist Binding to the GABAa or Benzodiazepine Sites by In Vivo Hormone Treatments[a]

Hormone	Area	Ligand	Binding	References
EB	POA/VMN/S/CTX	muscimol	↓	O'Connor et al., 1988
EB	VMN/MCG	muscimol	↓	Schumacher et al., 1989b
EB	VMN/POA/AMYG	muscimol	↑	Canonaco et al., 1989
EB	HYP/CTX/STR	muscimol	↑	Perez et al., 1986
EB	HYP	muscimol	↑	Lasaga et al., 1988
EB	CA1	muscimol	↑	Schumacher et al., 1989a
P[b]	VMN/MCG	muscimol	↑	Schumacher et al., 1989b
P	CTX	flunitrazepam	↑	Gavish et al., 1987
P	CP/POA/VMN	muscimol	↑	Canonaco et al., 1989
P	SUB. GEL.	flunitrazepam	↑	Schwartz-Giblin et al.,1988
CORT	CTX/HYP	Ro15-1788	↓	Miller et al., 1988
ADX	CTX, HIP/HYP	Ro15-1788	↑	Miller et al., 1988
ADX	CTX, HIP	muscimol	↓	Majewska et al., 1985
ADX	HYP	muscimol	↑	Majewska et al., 1985
ADX	HYP, STR	flunitrazepam[c]	↑	Goeders et al., 1986
ADX	HIP	flunitrazepam[c]	↓	Goeders et al., 1986

[a]EB = estradiol benzoate; P = progesterone; CORT = corticosterone; ADX = adrenalectomy. Binding has been quantified within different regions of the brain or spinal cord: AMY = amygdala; CAl = CAl region of the dorsal hippocampus; CP = caudate putamen; CTX = cortex; HIP = hippocampus; HYP = hypothalamus; MCG = midbrain central gray; POA = preoptic area; S = septum; STR = striatum; SUB GEL = substantia gelatinosa; and VMN = ventromedial nuclei of the hypothalamus.
[b]GABA stimulated.
[c]After estrogen priming.

different experimental conditions and may provide interesting information concerning the mechanisms of hormone-GABAa receptor interactions.

Thus, different concentrations of muscimol have been used in both studies, suggesting that estrogens may regulate the different affinity states of the GABAa receptor in a specific manner. That is, GABAa receptor binding within the cortex appears not to be affected by estrogen treatment when brain sections were incubated in the presence of low concentrations (5 n*M*) of (^{3}H)muscimol, but appears to be decreased when receptors were labeled with higher concentrations of ligand (20 n*M*). Because 5 n*M* of muscimol selectively label high-affinity GABAa sites, whereas 20 n*M* also label part of the low-affinity receptors in conditions used for receptor autoradiography (Fig. 4), these results suggest that estrogens may only affect low-affinity GABAa receptor binding in the cortex.

There are other differences for reported estrogen effects on GABAa receptors. Whereas two studies report a decrease of muscimol binding within the VMN after estrogen treatment (O'Connor et al., 1988; Schumacher et al., 1989b), other authors have found estrogens to increase muscimol binding within the same brain region of the hamster (Canonaco et al., 1989) or rat (Maggi and Perez, 1986; Perez et al., 1986, Lasaga et al., 1988). This discrepancy may reflect differences between species in the former case and different experimental conditions in the latter case. In these studies, female rats have been ovariectomized before puberty, and GABAa receptor binding has been measured in membrane preparations and not tissue sections, as for receptor autoradiography.

As is the case for estrogens and progestagens, the actions of glucocorticoids on the GABAa receptor are complex. They are dependent on the brain region and are sometimes biphasic, with

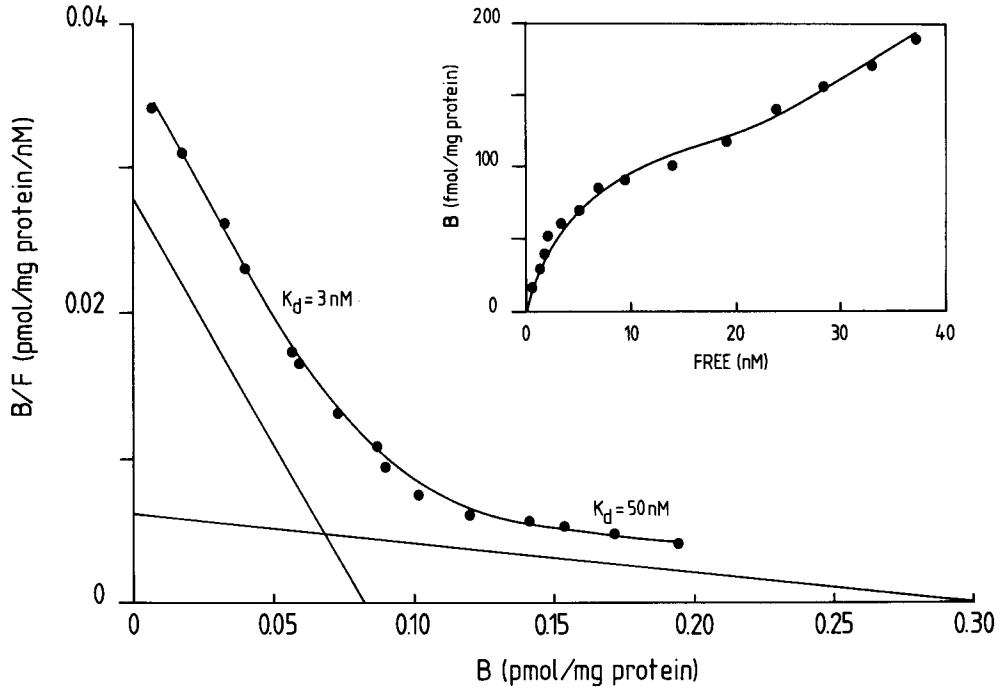

Fig. 4. Saturation isotherm (insert) and Scatchard plot of (^{3}H)muscimol binding to 14 m thick sections (11 mm diameter) cut from a whole brain paste in a cryostat and thaw-mounted onto glass slides. The assay conditions were the same as for receptor autoradiography except that, after incubation in the presence of different concentrations of ligand, the labeled paste sections were wiped from their glass slides with filter paper. Nonspecific binding was determined in the presence of 10 *M* cold GABA (modified from Schumacher et al., 1989b).

respect to their concentration. Thus, adrenalectomy decreases muscimol binding in hippocampus and cortex by reducing the affinity of the GABAa recognition site, but increases muscimol binding in the hypothalamus (Majewska et al., 1985). Steroid hormone treatments not only affect the GABA recognition site, but also the other drug-binding sites. That is, chronic treatment of rats with P increases the density of BZ binding sites in cerebral cortex without affecting their affinity (Gavish et al., 1987). Acute treatment of ovariectomized rats with P also increases flunitrazepam binding within the substantia gelatinosa of the spinal cord. This effect of P was decreased when rats were pretreated with estrogen (Schwartz-Giblin et al., 1988). Adrenalectomy also increases BZ binding in cortex, hippocampus, and hypothalamus by increasing B_{max}, without affecting the K_d. This effect can be reversed by corticosterone treatment (Miller et al., 1988).

There are several mechanisms through the mediation of which steroid hormones may affect agonist and drug binding to the GABAa receptor. Thus, steroids may modulate the GABAa receptor indirectly through the mediation of other regulatory factors. That is, chronic estrogen treatment has been reported to decrease muscimol binding to membranes from hippocampus, striatum, and cerebral cortex of intact female rats, but not hypophysectomized female rats. Results of this study suggest that pituitary prolactin may be involved in the estrogen mediated downregulation of brain GABAa receptors

(Hamon et al., 1983). Similarily, the P-induced increase in flunitrazepam binding at the level of the substantia gelatinosa of the spinal cord is prevented by spinal transection, suggesting that the P effect depends on supra-spinal input (Schwartz-Giblin et al., 1988).

One mode of action of steroid hormones is the regulation of genomic activity, which leads to the synthesis of specific gene products, including enzymes and receptors (McEwen et al., 1984, 1988). By regulating the transcription, and eventually the translation of mRNA coding for different subunits of the GABAa receptor in a site-specific manner, steroid hormones may modulate the number of different GABAa receptor subtypes within distinct brain regions. Since clones corresponding to the different subunits of the receptor complex are becoming available, this possibility can now be tested experimentally. That is, GABAergic neurons in the hypothalamus contain estrogen (Flügge et al., 1986) and progesterone (Leranth et al., 1988) receptors. Moreover, glutamic acid decarboxylase (GAD) immunoreactive axons end on progestin receptor-immunopositive hypothalamic neurons (MacLusky et al., 1988). Alternatively, as described below, steroid hormones have also been shown to influence the excitability of neurons by acting directly on the cell membrane, and they may do so by modulating the GABAa receptor. This has been suggested by the observations that alphaxalone (5α-pregnan-3α-ol-11,20-dione) increases the duration of inhibitory postsynaptic conductance in neurons of the guinea pig olfactory cortex (Schofield, 1980), enhances GABA-stimulated chloride conductance in the rat brain (Harrison and Simmonds, 1984), and that naturally occurring metabolites of P, which are characterized by a 3α-hydroxyl group and a 5α-reduced A-ring, produce rapid anticonvulsant effects by acting on neuronal membranes (Craig, 1966; Gee, 1988). Moreover, estrogens and progestagens affect muscimol binding in brain regions, like the cerebellum and the cortex, are devoid of measurable levels of intracellular steroid receptors (Perez et al., 1986; O'Connor et al., 1988).

In Vitro Effects of Steroid Hormones

As indicated above, certain steroids exert rapid effects on the GABAa receptor, possibly by acting directly on the neuronal membrane. That is, the A-ring reduced metabolites of progesterone (3α-hydroxy-5α-pregnane-20-one; 3α-OH-DHP) and deoxycorticosterone (5α- pregnane-3α, 21-diol-20-one; 3α-THDOC), which are inactive at the intracellular steroid receptors (Gee et al., 1988), have been shown to exert direct effects on the GABAa receptor. They do so by increasing the binding of muscimol and flunitrazepam and potentiating the GABA-mediated chloride ion uptake. They also inhibit TBPS binding in an uncompetitive manner (Majewska et al., 1986; Harrison et al., 1987; Morrow et al., 1987). Autoradiographic studies indicate that all detectable TBPS binding sites are sensitive to 3α-OH-DHP although there are regional differences in the potency of the hormone in modulating them (Gee et al., 1988). Physiological doses of GABA potentiate the inhibitory effects of 3α-OH-DHP and 3α-THDOC on TBPS binding (Gee et al., 1987, 1988). Thus, there is a reciprocal positive cooperativity between the GABA- and steroid-binding sites: steroids potentiate the binding of GABA, and GABA, in turn, enhances the effects of steroids.

The effects of 3α-OH-DHP and 3α-THDOC are similar, but not identical to those of the barbiturates. Like barbiturates, both steroids increase the number of high-affinity GABAa recognition sites (Peters et al., 1988), enhance the affinity of BZ sites (Majewska et al., 1986), and accelerate the dissociation of TBPS (Gee et al., 1988). Both 3α-OH-DHP and 3α-THDOC also potentiate the actions of GABA on membrane currents by enhancing chloride channel conductance (Callachan et al., 1987) and prolonging the open time of the chloride channel (Barker et al., 1987; Peters et al., 1988). Even in the absence of GABA, these steroids stimulate a slow inward chloride ion current, suggesting a direct link between the steroid acceptor site and the chlo-

ride channel (Majewska et al., 1986). Thus, like barbiturates, 3α-OH-DHP and 3α-THDOC increase the number of high-affinity GABAa receptors that are functional and show a high affinity for BZ and a very low affinity for convulsants. According to the "5-State Model" (*see* Fig. 2), these steroids may stabilize an active high-affinity conformation of the GABAa receptor that would correspond to State 4. However, whether steroid hormones exert their effects on the GABAa receptor by causing conformational changes of the protein still has to be explored. Before asserting that 5α-steroids shift the conformation of the receptor from a low-affinity state to high-affinity ones, it has to be shown that these hormones increase high-affinity muscimol binding at the expense of low-affinity sites and that they decrease the binding of antagonists, like (+)bicuculline, that selectively label low-affinity sites. 5α-Steroids have been reported to increase the number, rather than the affinity, of muscimol binding sites (Peters et al., 1988). However, in this particular study, bound and free radioligand were separated by filtration, and this is not an optimal method to detect low-affinity GABAa receptor sites. The fact that 5α-reduced steroids may indeed affect the affinity of the GABAa site has been suggested by another study. Tetrahydroprogesterone (5α-pregnane-3α-ol-20-one; THP) has been shown to increase muscimol binding to membranes prepared from rat forebrains by increasing the affinity of the GABA site. Similarily, during pregnancy, when plasma P and THP levels are high, the affinity of the GABAa receptor for muscimol is increased, but not the number of binding sites. This increase in muscimol binding is associated with a potentiation of GABA functions (Majewska et al., 1989).

Whether 5α-reduced steroids modulate the GABAa receptor by binding to a specific site on the macromolecular complex is still unknown. However, this is suggested by the structural requirements for activity and the nanomolar potencies of these steroids (Harrison et al., 1987; Gee et al., 1988). However, like a number of other receptors, the GABAa receptor complex is very sensitive to its lipid environment (Bristow and Martin, 1987). As a consequence, steroid hormones may exert their effects on the receptor without binding to it, but simply by modifying the fluidity of the surrounding cell membrane. Similar mechanisms of steroid hormone action have already been proposed for other transmitter receptors (Heron et al., 1980; Ciofalo, 1981). That is, 5α-reduced steroids are highly lipophylic, and membrane lipids are able to bind steroids (Fesik and Makriyannis, 1986). Such a mechanism of steroid action is not in contradiction with the observation of stereospecificity. Thus, 5α-pregnan-3α-ol-20-one and 5α-pregnan-3β-ol-20-one do differ in the degree of disorder that they induce in model membranes, with 3α-steroids being the most efficient in this respect (Lawrence and Hill, 1975). Whatever the mechanism of steroid action may be, there are similarities between the actions of steroids and barbiturates, and it is likely that both modulate the GABAa receptor by stabilizing an active high-affinity conformation. However, interactions between 3α-OH-DHP and pentobarbital in the potentiation of flunitrazepam binding, in reducing TBPS binding or activating transmembrane currents, show that steroids and barbiturates do not act at a common site (Lambert et al., 1987; Peters et al., 1988; Gee et al., 1988; Gee, 1988).

Steroids other than 3α-OH-DHP and 3-α-THDOC also exert direct effects on the GABAa receptor complex. Thus, nanomolar (n*M*) concentrations of corticosterone enhance muscimol binding to membrane preparations from the cortex and cerebellum by increasing the affinity of the GABAa binding site, without affecting its number (Majewska et al., 1985). This result fits well with the observation that adrenalectomy decreases muscimol binding in these brain regions. At low nanomolar concentrations, glucocorticoids also potentiate the binding of TBPS by increasing both the affinity and density of the binding site (Majewska, 1987a). By enhancing TBPS binding, glucocorticoids could increase neuronal excitability. Indeed, this has been shown by electrophysiological experiments (Feldman et al., 1961; Riker et al, 1982) and is in agreement with the observation that

glucocorticoids lower the threshold for seizures (Woodbury; 1958, Majewska, 1987a). If corticosteroids exert these effects by changing the conformation of the GABAa receptor, then our "5-State Model" would predict that these hormones would shift the conformation of the receptor to an inactive, high-affinity state since not only GABA agonist, but also convulsant binding are increased (State 3).

There is a particular class of steroid hormones that may also modulate activity GABAa receptors. These hormones, dehydroepiandrosterone (D), dehydroepiandrosterone sulfate (DS), as well as their precursors, pregnenolone (P), and pregnenolone sulfate (PS), are called "neurosteroids" because they are produced by the brain itself (Corpéchot et al., 1981, 1983; Hu et al., 1987). Neurosteroids have been reported to reduce neuronal and glial death and improve memory functions (Bologna et al., 1987; Roberts et al., 1987; Flood and Roberts, 1988). Several studies suggest that neurosteroids may exert their effects on brain cells by acting directly on the GABAa receptor. However, their mechanism of action is much debated. Some studies have suggested that PS may competitively bind to the TBPS binding site and, thus, mimic the effects of this convulsant. That is, PS has been shown to inhibit muscimol-stimulated chloride ion uptake in brain synaptosomes and antagonize pentobarbital-stimulated flunitrazepam binding at low micromolar concentrations (Majewska and Schwartz, 1985). These inhibitory effects of PS on the chloride ionophore are in agreement with the observation that D, DS, and PS exert excitatory effects on the neuronal membrane when applied iontophoretically to cells in the septopreoptic area (Carette and Poulain, 1984). Similarily, micromolar concentrations of PS reversibly inhibit GABA-generated currents in isolated cortical neurons in a manner similar to picrotoxin (Majewska et al., 1988). That is, both PS and picrotoxin decrease the opening frequency of the ion channel (Mienville and Vicini, 1989). However, another recent study suggests that PS is not competitive with the TBPS site. That is, the effect of PS is to accelerate the dissociation of TBPS, an effect that is potentiated by GABA (Gee et al., 1989). Whatever, the concentrations of PS required to inhibit TBPS binding are in the micromolar range, and levels of PS in the brain appear to be 2–3 orders of magnitude less (Corpéchot et al., 1983). Consequently, the effects that PS exerts on TBPS binding in vitro may not be physiologically relevant (Gee et al., 1988). However, nanomolar concentrations of PS have been shown to enhance muscimol binding (Majewska et al., 1985; Majewska, 1988), and some studies suggest that PS may potentiate GABA effects in a manner consistent with the action of 5α-reduced, 3α-ol steroids (Gee et al., 1988, 1989). That is, PS has been reported to have anticonvulsant effects when administered in vivo (Gee, 1988; Gee et al., 1988).

Significance of the Steroid–GABAa Receptor Interactions

The potential therapeutic applications of A-ring-reduced steroids have been reviewed recently (Gee, 1988). These involve the treatment of catamenial epilepsy and premenstrual syndrome (PMS). Since steroid hormones modulate the binding of drugs to the GABAa receptor complex in an allosteric manner, it is not surprising that they interact with antiepileptic drugs. That is, the incidence of seizures increases during periods of the menstrual cycle when levels of circulating P are low (Backstrom, 1976; Backstrom et al., 1985). Similarily, the number of seizures in women with catamenial epilepsy is lowest when circulating progesterone reaches its highest levels (Rosciszewska et al., 1986). These observations are in agreement with the barbiturate-like (= anticonvulsant) effects of progestagens. Emotional symptoms related to PMS are also correlated with reduced P levels (Backstrom et al., 1985). Recent studies show that THDOC has sedative and anxiolytic properties in rodents (Crawley et al., 1986; Mendel-

son et al., 1987). Thus, the effectiveness of treatments with barbiturates or benzodiazepines is, in part, dependent on the endocrine status of the patient. One therapeutic strategy may be to administer steroid hormones during periods of reduced hormone levels (Gee, 1988) or prior to the administration of anxiolytic, analgesic, or sedative GABAergic drugs in order to increase their efficacity or even their specificity. However, the development of such combined treatments requires a better understanding of the mechanisms through the mediation of which steroid hormones modulate, in an allosteric manner, specific GABAa receptor subtypes.

Conclusions

Steroid hormones modulate the GABAa receptor when administered in vivo. They may do so by regulating the expression of genes that code for the different receptor subunits. This possibility has not been explored so far. However, probes for the different genes that code for the different receptor subunits are now available, and their regulation by hormones can be studied within distinct brain regions by *in situ* hybridization histochemistry. Steroid hormones also exert rapid effects on the GABAa receptor by binding directly to the receptor or modifying its lipid environment. According to the various models of the GABAa receptor (Figs. 1 and 2), binding of a drug to its specific site causes the conformation of the receptor to change. As a consequence, the characteristics of the other binding sites, and the functional properties of the receptor complex, are modified. We propose that steroid hormones may act in a similar manner. By binding to the GABAa receptor or to specific membrane acceptor sites that are located close to the receptor, gonadal and adrenal steroids may shift the conformation of the GABAa receptor between active and inactive states. 5α-Reduced steroids, like 3α-OH-DHP and 3α-THDOC, may, like barbiturates, stabilize an active receptor conformation, which is characterized by high-affinity GABA and BZ sites and low-affinity convulsant sites. This conformation would correspond to State 4 of our "Five-State Model" (Fig. 2). By contrast, corticosteroids may favor a high-affinity inactive conformation of the receptor (State 3, Fig. 2) since these hormones increase the affinity of the GABA site and, possibly, the convulsant site for their agonists. At least at low nanomolar concentrations, neurosteroids, like PS, may exert similar effects than the 5α-reduced metabolites and, thus, potentiate the active fourth conformation of the GABA receptor.

Discussion

In the present review, we have described three basic mechanisms through the mediation of which steroid hormones may modulate the GABAa receptor in neural tissue. First, they may do so by acting directly on the receptor or its environment, changing its conformation. These conformational changes may depend on the subunit composition of the receptor complex. Second, they may also affect the synthesis and expression of different receptor subunits by regulating the activity of their corresponding genes in a specific manner. Third, steroid hormones may modulate the GABAa receptor, in an indirect manner, through the mediation of other regulatory factors. Thus, by changing the activity of protein phosphorylating enzymes or modifying the synthesis of regulatory peptides, like prolactin, steroid hormones may regulate GABAa receptor binding. This possibility has not been explored extensively although some evidence pertaining to prolactin involvement was noted earlier. However, we have discussed evidence that the first two mechanisms are involved in the interactions between steroids and the GABAa receptor. Specific agonists and antagonists, as well as the clones for the different subunits of the GABAa receptor, are now available and shall allow us to further investigate the effects of steroids. Finally, the central GABAa

receptor is desensitized or downregulated by its neurotransmitter or a chronic exposure to benzodiazepines (Gallager et al., 1984; Schwartz et al., 1986; Cash and Subbarao, 1987; Tehrani et al., 1988). Thus, in vivo administration of GABAergic drugs and steroid hormones may also modulate GABAa receptor binding by altering the release rates of GABA or endogenous benzodiazepines. That is, estrogens have been shown to affect levels and turnover of GABA, as well as the activity of GABA-metabolizing enzymes in specific hormone-sensitive brain regions (McGinnis et al., 1980; Mansky et al., 1982; Duvilanski et al., 1983; Frankfurt et al., 1984).

We have proposed that drugs and steroid hormones may modulate the activity of the GABAa receptor complex by changing its conformation. According to our "Five-State Model" (Fig. 2), the GABAa receptor may adopt two additional active, high-affinity conformations in addition to the three recognized previously. The new model fits most of the pharmacological and electrophysiological data that are available. However, it does not explain the heterogeneity of the central GABAa receptor. This heterogeneity may result from a different subunit composition of different receptor populations. Different receptor populations may also explain why steroid hormones exert different effects on the receptor within different brain regions. That is, different GABAa receptor populations may show different sensitivities to steroids. Investigating the regulation of specific GABAa receptor subtypes by steroid hormones at the genomic and membrane levels within distinct brain regions is now possible using the techniques of in vitro receptor autoradiography and *in situ* hybridization histochemistry. In the near future, such studies may lead to the development of new therapeutic strategies, including the combined administration of steroids and GABAergic drugs.

Although our "Five-State model" does not explain all experimental observations, it constitutes an useful working model for further investigations. That is, it allows us to predict the consequences of the binding of a single drug, ion, or hormone to the receptor complex on the characteristics of the other recognition sites and their complex interactions. The model also allows speculation about the functional significance of drug and hormone actions on the GABAa receptor. Thus, an increase in muscimol binding by a given hormone may correspond to an increase in the number of GABAa sites or the stabilization of an inactive or active high-affinity state. Regarding the great number of drug binding sites that reside on the receptor complex (six or even more), it is possible that some GABAa receptors may even adopt more than five distinct conformations. That is, the present model does not explain the actions of some drugs, like avermectines, that enhance BZ binding and decreases GABA binding (Olsen and Snowman, 1985). However, before describing supplementary interconvertible conformations of the GABAa receptor, it is necessary to obtain more experimental data.

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