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COMMENTARY

AN ALLOSTERIC MODEL FOR BENZODIAZEPINE RECEPTOR FUNCTION

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The assignment of specific ^{3}H-labeled ligand binding to a pharmacologically relevant receptor is not a trivial matter. Nevertheless, evidence has accumulated during the past few years that benzodiazepines mediate their pharmacological effects by interacting with neuronally localized receptors within the brain. An intriguing question is how the recognition of a benzodiazepine by its receptor initiates a chain of events leading to the pharmacological response, e.g. prevention of seizures, tranquility and sedation. The conventional approach has been to rationalize the initial recognition step within the context of a lock-and-key model. This hypothesis can readily account for the pharmacological specificity and stereospecificity of receptors as being a consequence of their geometry. It is with this recognition process that the implications of receptor binding data have their most immediate application. In addition, ligand binding methods can yield some insight into the activation of the receptor–effector system. It has been pointed out that part of the intrinsic binding energy of an agonist is used to induce a conformational change in the receptor [1]; in other words, the observed affinity of a drug for a receptor depends on both the bimolecular association of the ligand with the receptor and the subsequent conformational change of the ligand–receptor complex. Although this idea is not a new one, the importance of this phenomenon in regard to interpreting binding data is not generally recognized.

It is useful to consider how the binding properties of drugs for other neurotransmitter receptors are affected by the intrinsic activity of the ligand. With regard to beta-adrenergic and muscarinic receptors, there are striking differences in the way agonists and antagonists bind to these receptors [2–4]. In general, agonists tend to display heterogeneous binding properties [5]. The magnitude of this heterogeneity can be estimated by computing the relative differences in the affinities of a drug for subclasses of the receptor or, in some instances, by estimating the relative densities of the high and low affinity states of the receptor. Strong correlations between the heterogeneity of binding and the efficacy of the ligand have been noted for muscarinic and beta-adrenergic receptors [2–4]. Also, the negatively cooperative effects of guanine nucleotides on binding properties show a strong correlation with efficacy [5, 6]. For opiate receptors, a correlation between the influence of Na^+ on binding and the efficacy of opiate analogues has been noted [7]. It is not our intent to assess the models that have been proposed to account for these phenomena but merely to emphasize the fundamental principle providing the basis for these relationships: *the intrinsic activity of an agonist is related to its ability to induce a conformational change in the receptor.*

During the course of our studies of the benzodiazepine receptor binding activity of the beta-carboline compounds which are structurally unrelated to benzodiazepines (see Fig. 1), we noticed certain correlations between the influence of gamma-aminobutyric acid (GABA) on binding and the intrinsic activity of the drug [8, 9]. These differences have been rationalized in terms of an allosteric model for benzodiazepine receptor function which views the benzodiazepine receptor as a heterotropic site on the GABA receptor–chloride ionophore complex [10]. The model is an attempt to define the relationships between the behavioral effects of benzodiazepines and the influence of GABA on binding. Presently, it is our goal to describe the model in greater detail and to demonstrate that it can account for most of the pharmacological effects of ligands that interact specifically with the recognition site of the benzodiazepine receptor. We shall begin our discussion by reviewing some of the complex binding characteristics of the benzodiazepine receptor.

Benzodiazepine receptor heterogeneity

Initially, it seemed as if benzodiazepine receptors were homogeneous [11–13]. This assumption was based on the results of numerous binding studies which have shown that the equilibrium binding isotherms for [^{3}H]diazepam and [^{3}H]flunitrazepam ([^{3}H]FLU) in the range of concentrations studied were consistent with the simple Langmuir isotherm [11–15]. Also, when the specific binding of [^{3}H]FLU or [^{3}H]diazepam in low concentrations is inhibited by other nonlabeled benzodiazepines, the competition curves usually have Hill coefficients of approximately 1. However, evidence soon emerged which suggested that benzodiazepine receptors are not simply a homogeneous class of binding sites. In 1979,

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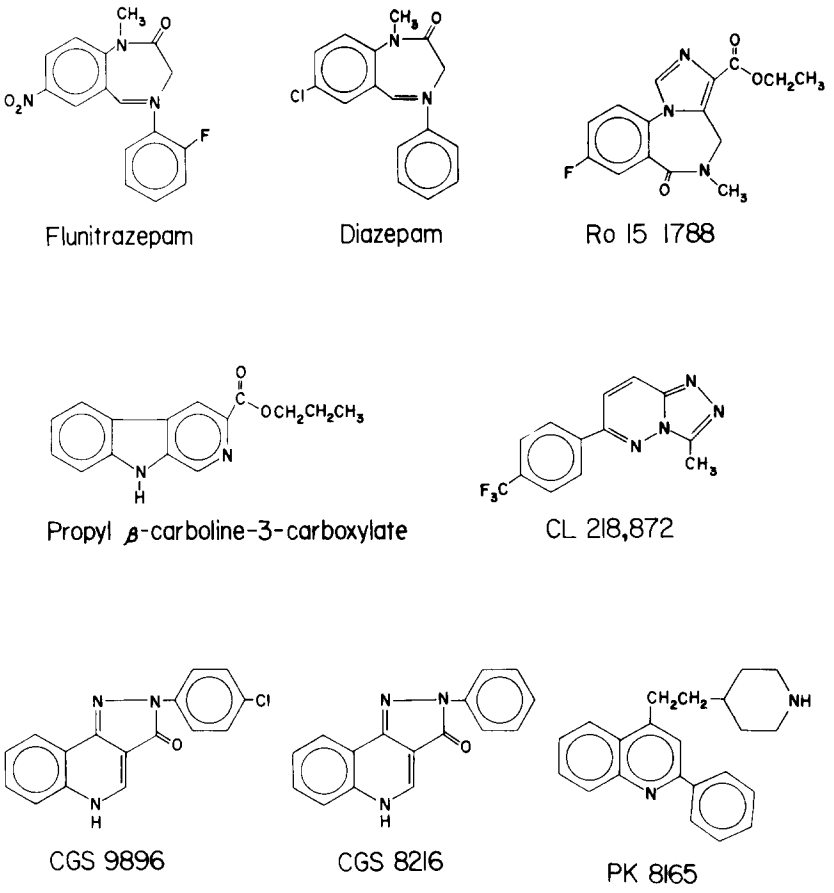

Fig. 1. Structures of some benzodiazepine receptor ligands referred to in the text.

Squires *et al.* [16] found that a new class of compounds, the triazolopyridazines, inhibited [^{3}H]FLU binding in a manner characterized by Hill coefficients of approximately 0.7. One member of the triazolopyridazines, CL 218,872, was found to be an effective inhibitor of [^{3}H]diazepam binding (IC_{50} = 67 nM) [16] and to prevent both metrazol-induced convulsions and behavior reinforced by punishment (anticonflict test) at doses comparable to those of diazepam [17]. However, unlike the benzodiazepines, CL 218,872 did not cause sedation of ataxia at doses ten times those that were effective in the anticonflict test [17]. Thus, the triazolopyridazines were proposed to represent a selective class of anxiolytic, anticonvulsant drugs that lack the sedative properties of benzodiazepines.

Complexities in the nature of benzodiazepine receptor binding have been noted with another class of compounds, the beta-carbolines. The methyl, ethyl and propyl esters of beta-carboline-3-carboxylic acid (MCC, ECC and PCC) are potent inhibitors of [^{3}H]FLU binding, with IC_{50} values in the nanomolar range [10, 18, 19]. Moreover, the competition curves of PCC, ECC and MCC for inhibiting [^{3}H]FLU binding are shallower than a mass-action curve and, consequently, the Hill coefficients are less than 1 [10, 18, 19]. However, unlike the benzodiazepines, alkyl esters of beta-carboline-3-carboxylate have pharmacological properties that are pharmacologically antagonistic to the tranquilizing 1,4-benzodiazepines [20–24].

The complex natures of the binding of the triazolopyridazines and the alkyl beta-carboline-3-carboxylates have been rationalized in terms of benzodiazepine receptor heterogeneity. In general, the data have been consistent with the presence of two major classes of benzodiazepine receptors known as the type I and type II receptors [25–27]. The alkyl esters of beta-carboline-3-carboxylate (ECC and PCC) and the triazolopyridazine, CL 218,872, are thought to discriminate between the type I (high affinity) and type II (low affinity) receptors, whereas the 1,4-benzodiazepines have nearly equal affinity for both types of receptors. The results of the PCC/[^{3}H]FLU competition curve shown in Fig. 2 are consistent with this interpretation of benzodiazepine receptor heterogeneity. It can be seen that the competition curve is flatter than a mass-action curve as indicated by the systematic deviations between the data points and the dotted line which represents a mass-action curve through the IC_{50} point. In contrast, there is good agreement between the data points and the solid curve; the latter represents the best fit to the data assuming two binding sites for PCC, a high affinity site (K_H = 0.5 nM) and a low affinity site (K_L = 10 nM) having relative abundances of 55 and 45% respectively. The proportion of benzodiazepine receptors that are type I varies in different regions

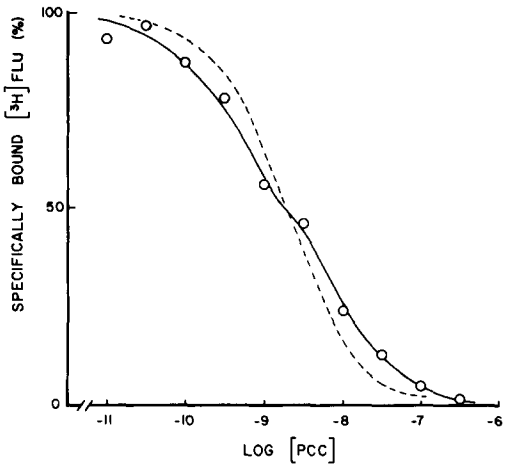

Fig. 2. Competitive inhibition of [^{3}H]FLU binding by PCC. Assays were run on the rat cerebral cortex at 0°, and binding was measured at a [^{3}H]FLU concentration of 0.5 nM. Other experimental details are given in Ref. 10. The data points represent mean specific [^{3}H]FLU binding values determined in the presence of increasing concentrations of PCC. The dotted line represents a mass-action curve through the IC_{50} point on the curve. The solid line represents the best fit to the data, assuming two major binding sites for PCC.

of the brain. The cerebellum contains predominantly type I receptors; the cerebral cortex has an intermediate proportion of about 50%; the hippocampus contains a small proportion of type I receptors [10, 19, 27–29].

In general, direct measurements of the binding of [^{3}H]beta-carbolines provided data that can also be rationalized in terms of benzodiazepine receptor heterogeneity. It has been noted that the maximum number of binding sites for [^{3}H]PCC is equivalent to that of [^{3}H]FLU in the cerebellum but is somewhat smaller in the hippocampus, suggesting that [^{3}H]PCC labels predominantly type I receptors at the ^{3}H-labeled ligand concentrations usually employed in binding assays [27]. When PCC receptor occupancy was estimated by direct measurements of [^{3}H]PCC binding and by PCC/[^{3}H]FLU competition, there was general agreement between the two independent methods suggesting that the deviations from the simple Langmuir isotherm seen in PCC/[^{3}H]FLU competition experiments are caused by receptor heterogeneity and not complex ligand interactions between [^{3}H]FLU and PCC [10].

The results of ligand/[^{3}H]alkyl beta-carboline-3-carboxylate competition experiments are consistent with the concept of benzodiazepine receptor heterogeneity. When low concentrations of ^{3}H-labeled ligands are used, the potencies of PCC, ECC and MCC are much greater when measured by inhibition of [^{3}H]alkyl beta-carboline-3-carboxylate binding as compared to that measured by inhibition of [^{3}H]FLU binding [10, 30]. These data are consistent with the idea that [^{3}H]alkyl beta-carboline-3-carboxylates bind selectively to the high affinity, type I receptor at low ligand concentrations, whereas [^{3}H]FLU does not show this selectivity. In contrast, the potency of FLU is approximately the same when measured by inhibition of [^{3}H]PCC or [^{3}H]FLU binding, consistent with the idea that [^{3}H]FLU has equal affinity for type I and type II receptors [10].

We have noticed additional complexities in the nature of beta-carboline binding that cannot be rationalized on the basis of only two types of benzodiazepine receptors. When low concentrations (0.05 nM) of tritiated PCC, ECC and MCC are used, and binding is inhibited by the same nonlabeled alkyl beta-carboline-3-carboxylate, the competition curves deviate significantly from the simple Langmuir isotherm and are consistent with the presence of a small proportion of super high affinity sites (K_D 30–50 pM) that represent only 3–6% of the total number of benzodiazepine receptors in the cerebral cortex [10].

On the basis of changes in benzodiazepine receptor binding properties following photolabeling of membranes from mouse brain with nonlabeled FLU, Hirsch and co-workers [31, 32] have suggested that "beta-carboline binding sites" are distinct from benzodiazepine binding sites. In the foregoing studies, cerebellar, hippocampal and striatal membranes were irradiated with ultraviolet light in the presence of FLU and, subsequently, an 80% decrease in [^{3}H]FLU binding was noted while only a 15% decrease in the binding of [^{3}H]PCC was observed. In spite of the foregoing evidence, it is difficult to rationalize the concept of distinct "beta-carboline binding sites" for the following reasons. In control membranes, the K_i value and maximal inhibition of ^{3}H-labeled ligand binding are approximately the same for FLU when measured by inhibition of specific [^{3}H]PCC binding or [^{3}H]FLU binding. Thus, if PCC and FLU were to bind at distinct sites, then the negative cooperativity between the two sites would have to be very great so that the interaction between these ligands would appear competitive. Such an interaction would require a large PCC-induced alteration in the kinetics of [^{3}H]FLU binding so that the dissociation constant of FLU would appear to be much greater (lower affinity) in the presence of excess PCC (10^{-6} M). However, a large PCC-induced increase (30- to 100-fold) in the dissociation kinetics of [^{3}H]FLU binding has never been reported, and it seems unlikely that such a phenomenon is possible. Interestingly, it has been noted that photolysis of cortical membranes in the presence of [^{3}H]FLU leads to an irreversible alkylation of only 25% of the receptors with [^{3}H]FLU while the remaining 75% of the receptors lose their ability to bind [^{3}H]FLU [33]. If beta-carbolines and benzodiazepines stabilize the benzodiazepine receptors in different conformations, it seems possible that the remaining 75% of the photolized receptors that are not bound irreversibly with FLU might still retain their ability to bind beta-carbolines. Mohler [34] has expressed a similar view in a recent report.

Allosteric model for benzodiazepine receptor function

It is difficult to construct a model for benzodiazepine receptor function solely on the basis of the complex interactions of ligands with the heterogeneous population of benzodiazepine receptors. The reason for this dilemma is the lack of correlation between the heterogeneity of binding and the pharmacological activities of drugs which interact with

the benzodiazepine receptor. For example, the alkyl beta-carbolines (MCC and ECC) and the triazolopyridazine, CL 218,872, all display heterogeneous binding properties [16, 18, 19, 35], yet MCC and ECC antagonize the effects of the tranquilizing benzodiazepines [20–24, 36] while CL 218,872 has pharmacological effects similar to the benzodiazepines [17]. Moreover, the imidazodiazepine Ro15 1788 has a homogeneous binding profile like FLU and diazepam [37], yet it specifically antagonizes the effects of benzodiazepines in a variety of behavioral and electrophysiological experimental paradigms [38]. Thus, there is no apparent correlation between the heterogeneity of binding and the intrinsic activity of drugs that interact with the benzodiazepine receptor.

We initially proposed that the efficacy of benzodiazepine receptor-ligands might be correlated with the influence of GABA on binding affinity in a study comparing [^{3}H]PCC with [^{3}H]FLU [8]. The concept has been extended to all compounds that interact with central benzodiazepine receptors [9, 10] and has also been confirmed by others [39]. There is ample evidence implicating the involvement of GABA mechanisms in the pharmacological effects of benzodiazepines. Benzodiazepines have been shown to potentiate the electrophysiological effects of GABA in a variety of neuronal systems (see review by Tallman *et al.* [40]). What might be considered as direct evidence for coupling between GABA receptors and benzodiazepine receptors was shown by copurification of soluble forms of both receptors [41, 42]. Also, in binding experiments, GABA enhances the affinity of benzodiazepines [43–46]. Several pieces of evidence indicate that chloride channels are associated with the benzodiazepine receptor–GABA receptor complex. Chloride has been shown to stimulate benzodiazepine receptor binding [47, 48], and this stimulation of binding is blocked by anion channel blockers [49]. Numerous interactions between agents that interact with GABA receptors, benzodiazepine receptors, and chloride channels have been described (see review by Olsen [50]) and, ultimately, these results lead to the suggestion that benzodiazepines mediate their pharmacological effects by interacting with a macromolecular complex consisting of a benzodiazepine receptor, a GABA receptor, and a chloride ionophore.

Because of the foregoing evidence, we sought to determine if a correlation between the GABA modulation of benzodiazepine receptor binding and the intrinsic activity of benzodiazepine receptor ligands could be demonstrated. Initial experiments were carried out on extensively washed membranes of the rat cerebral cortex, and binding assays were run at 37° in phosphate buffered saline (181 mM Na^+, 9.5 mM K^+, 100 mM Cl, 50 mM PO_4, pH 7.4). Under these conditions, we noticed that GABA produced a relatively large decrease (3-fold) in the K_i of FLU, and it seemed likely that we would be able to distinguish between ligands, depending upon whether GABA had a large, small, or undetectable effect on their binding. This degree of discrimination would not be expected at 0°, since the magnitude of the GABA effect on benzodiazepine receptor binding is less at 0°. Moreover, when assays are run at 37° in phosphate buffered saline, the temperature and ionic milieu more closely resemble physiological conditions and, thus, the implications of the results are more meaningful, particularly when correlations between binding properties and pharmacological activity are concerned.

On the basis of initial experiments with FLU and PCC, it was suggested that the influence of GABA on benzodiazepine receptor binding might correlate with the efficacy of the ligand [8, 10]. This postulate was based on the finding that GABA caused a large increase in the binding affinity of FLU while, under similar assay conditions, no significant effect of GABA on the binding of PCC was detectable [8, 10]. Previous experiments had shown that ECC antagonized the pharmacological effects of benzodiazepines [20–22], and it seemed quite likely that the propyl ester of beta-carboline-3-carboxylate might also share this pharmacological property. Since the initial studies, a good correlation between the GABA modulation of binding affinity and the efficacy of a structurally diverse group of drugs that interact with the benzodiazepine receptor has emerged [8–10, 29, 37, 51–54]. For example, the affinities of some novel anxiolytics including the triazolopyridazine CL 218,872, and the quinoline derivative PK 8165, are enhanced by GABA [9, 29, 51, 53], whereas the affinity of the benzodiazepine antagonist Ro15 1788 is unaffected by GABA [9, 37]. Moreover, the binding of the beta-carboline derivatives, ECC and MCC, has been shown to be either unaffected or inhibited by GABA [39, 55–57]. As mentioned previously, these compounds antagonize the effects of the tranquilizing benzodiazepines. An interesting pair of compounds is the pyrazoloquinoline CGS 8216 and its chloro derivative CGS 9896. Pharmacological experiments have shown that CGS 8216 antagonizes the effects of benzodiazepines [58] whereas its chloro derivative has benzodiazepine-like activity; CGS 9896 prevents both metrazol- and bicuculline-induced seizures [52]. Interestingly, GABA inhibits the binding of the benzodiazepine antagonist, CGS 8216, whereas the potency of the benzodiazepine-like derivative, CGS 9896, is enhanced by GABA [52, 53]. A summary of the effects of GABA on the benzodiazepine receptor binding activity of analogues is shown in Fig. 3. In general, the largest GABA-shift (ratio of K_i values in the absence and presence of GABA) is seen with benzodiazepines, like FLU, which display the full spectrum of pharmacological effects associated with benzodiazepines including tranquility, sedation, ataxia, and prevention against seizures. An intermediate GABA-shift is seen with the selective anticonflict (anxiolytic), anticonvulsant CL 218,872, whereas a small GABA-shift is seen with the selective anticonflict drug (anxiolytic), PK 8165 [59]. This quinoline derivative lacks the anticonvulsant and sedative properties of benzodiazepines [59]. In contrast, a GABA-shift of approximately 1 or less was observed with the benzodiazepine antagonists, PCC, Ro15 1788 and CGS 8216. Thus, the data in Fig. 3 show that GABA increases the potency of benzodiazepine-like compounds but has no effect on, or decreases the affinity of, benzodiazepine antagonists.

The most plausible interpretation for the effects

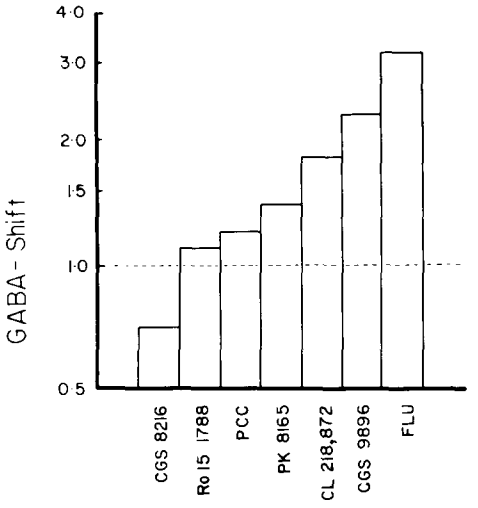

Fig. 3. Influence of GABA on the benzodiazepine receptor binding activity of a structurally diverse group of analogues. The competitive inhibition of [^{3}H]FLU binding by a variety of compounds was measured in the presence and absence of GABA. All assays were carried out on the rat cerebral cortex at 37° in phosphate buffered saline. The GABA-shift is defined as the ratio of K_i values of the competitive inhibitor measured in the absence and presence of GABA. The GABA-shift for CGS 8216 was determined by actual measurements of [^{3}H]CGS 8216 binding in the presence and absence of GABA. All assays were carried out using 10^{-5} M GABA (FLU, CL 218,872, PCC and Ro15 1788) or 10^{-4} M GABA (CGS 8216, CGS 9896 and PK 8165). Both of these concentrations of GABA produced similar maximal effects on the binding of several ligands. The data are from Refs. 9, 52 and 53. Statistical analysis showed that the "GABA-shifts" for PCC and Ro15 1788 were not significantly different from 1, whereas the "GABA-shifts" of the other compounds were significantly different from 1 ($P < 0.05$).

of GABA on the binding of benzodiazepines is that GABA binds at an allosteric site to induce a conformational change in the benzodiazepine receptor. Previously, we have applied the model shown in Fig. 4 to account for the heterotropic interactions between the binding of GABA and benzodiazepines [10, 60]. According to the model, the benzodiazepine receptor is part of a GABA receptor–chloride ionophore complex, and the complex is in equilibrium between ground (R) and activated (R^*) states associated with closed and open states of the chloride channel respectively. The equilibrium between the free forms of the complex can be described by the constant, K_4 (see equation 1 below; $K_4 = R^*/R$). In the absence of ligands the ground state of the complex predominates ($K_4 < 1$). GABA and benzodiazepines, like FLU, bind selectively to the activated state of the complex as indicated by the greater complimentariness between the benzodiazepine and GABA molecules and their respective receptors when the complex is in the activated state. The greater selectivity of these ligands for the activated state of the complex enables them to shift the equilibrium shown in Fig. 4 to the right. In other words, benzodiazepines such as FLU facilitate the postsynaptic effects of GABA so that there is a greater chloride flux when such benzodiazepines are present. It is proposed that the imidazodiazepine Ro15 1788 has equal affinity for the ground and activated states of the complex; thus, the binding of Ro15 1788 should be insensitive to GABA, and Ro15 1788 should antagonize the pharmacological effects of benzodiazepines. It has been shown that the binding of certain compounds including MCC and CGS 8216 is actually inhibited by GABA [39, 52, 55]. Braestrup *et al.* [39] and ourselves [53] have used the model (Fig. 4) to account for the inhibitory effect of GABA

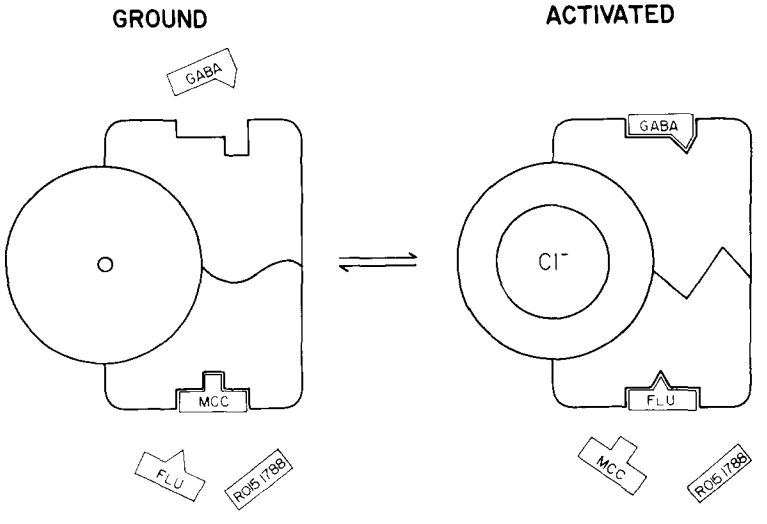

Fig. 4. Model for benzodiazepine receptor function. Benzodiazepines are thought to mediate their pharmacological effects on a macromolecular complex consisting of a chloride channel, a GABA receptor, and a benzodiazepine receptor. It is proposed that the complex can exist in two conformations, depending upon the nature of the ligands bound to it. In the absence of ligands, the ground state of the complex is preferred, and, in this conformation, the chloride channel is nonconductive. GABA and benzodiazepine stabilize the complex in an activated state characterized by an open chloride channel. Further details are given in the text.

on MCC binding. Accordingly, if it is assumed that the affinity of MCC is higher for the ground state of the complex than for the activated state (see Fig. 4), GABA should reduce the affinity of MCC for the benzodiazepine receptor. Such a model of negative heterotropic cooperativity would predict that the relationship between GABA and MCC is psuedo-competitive. Thus, MCC would not only antagonize the effects of tranquilizing benzodiazepines, but it would also tend to inhibit GABA transmission even in the absence of benzodiazepines.

If the allosteric model shown in Fig. 4 is an accurate interpretation of the influence of GABA on the benzodiazepine receptor, then GABA should alter the kinetics of [^{3}H]FLU binding. Such an effect was initially noted by Regan *et al.* [29] who investigated the influence of GABA on the dissociation kinetics of [^{3}H]FLU binding. In the absence of GABA, the dissociation kinetics of [^{3}H]FLU were characterized by fast and slow components. In the presence of GABA, the magnitude of the slow component was increased so that the overall dissociation kinetics were slower. On the basis of the dissociation kinetics of [^{3}H]FLU, Regan and coworkers [29] suggested that GABA induces a conformational change in the benzodiazepine receptor. In agreement with this suggestion, Chiu *et al.* [61] have proposed a square of equilibria involving two states of the benzodiazepine receptor to account for the kinetics of [^{3}H]FLU binding:

$$\begin{array}{lll} D_1 + R \overset{K_1}{\rightleftharpoons} D_1R & & \\ K_4 \downarrow\uparrow \qquad \downarrow\uparrow K_2 & & K_1K_2 = K_3K_4 \qquad (1) \\ D_1 + R^* \overset{K_3}{\rightleftharpoons} D_1R^* & & \end{array}$$

in which D_1 refers to FLU and R^* and R are high and low affinity states of the benzodiazepine receptor respectively. It is reasonable to assume that the contribution of the activated state of the receptor (R^*) is small compared to R ($K_4 < 1$) so that the equilibria can be approximated by the following "induced fit" model:

$$D_1 + R \overset{K_1}{\rightleftharpoons} D_1R \overset{K_2}{\rightleftharpoons} D_1R^* \qquad K_2 > 1 \qquad (2)$$

It should be noted that, at equilibrium, the consequences of the two-state model (equation 1) are the same regardless of whether the drug induces a conformational change in the receptor by the mechanism shown in equation 2 or whether the drug binds selectively to the activated state of the receptor as shown below:

$$\begin{array}{lll} D_1 + R \overset{K_1}{\rightleftharpoons} D_1R & & \\ K_4 \downarrow\uparrow & & K_4 < 1; K_1 < K_3 \qquad (3) \\ D_1 + R^* \overset{K_3}{\rightleftharpoons} D_1R^* & & \end{array}$$

All that is required is that ligands must have higher affinity for R^* if they are to induce a conformational change in the benzodiazepine receptor. The introduction of GABA binding to the receptor complex leads to the complicated system of equilibria shown in Fig. 5. The net effect is that, in the presence of both GABA (D_2) and benzodiazepines (D_1), a

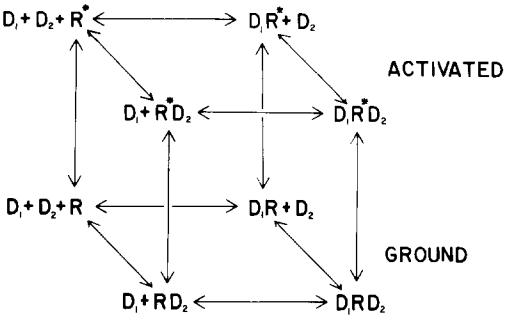

Fig. 5. Equilibrium between GABA (D_2), a benzodiazepine receptor ligand (D_1), and the macromolecular protein complex. The complex has two distinct binding domains and exists in two conformations (R and R^*), as described in Fig. 4.

greater proportion of the receptor assumes the activated conformation due to the inherent stability of the high affinity, ternary complex ($D_1R^*D_2$). This model predicts that benzodiazepines should enhance the binding of GABA to a receptor having an affinity constant that agrees with the potency of GABA for stimulating benzodiazepine receptor binding. It also predicts that the potency of GABA for stimulating the binding of drugs to the benzodiazepine receptor should be greater than that for inhibiting the binding of drugs (MCC) to the receptor.

The magnitude and quality (negative or positive) of the cooperativity between the binding of GABA and benzodiazepine receptor ligands can predict many of the behavioral effects of drugs that interact specifically with benzodiazepine receptors. It follows from the model in Fig. 4 that the extent to which GABA influences the binding of a drug to the benzodiazepine receptor is a measure of the extent to which the drug influences the neurotransmission of GABA and, ultimately, behavior. A compound like FLU, which shows a large GABA-shift, displays the full spectrum of behavioral effects of benzodiazepines, including anxiolytic effects at low doses and sedation and ataxia at higher doses. A compound like CL 218,872, which displays an intermediate GABA-shift, has anticonflict (anxiolytic) and anticonvulsant effects, yet it lacks the sedative and ataxia effects of the benzodiazepines [17]. Drugs that show a feeble GABA-shift would only cause a small increase in GABA transmission and, consequently, a compound of this nature would not be expected to display all the pharmacological effects of benzodiazepines. PK 8165 fulfills these criteria since it shows a small GABA-shift [53], and it is a selective anticonflict drug (anxiolytic) which lacks anticonvulsant, sedative and ataxia-producing properties [59]. For compounds having a GABA shift of less than 1, the model predicts that these compounds would actually antagonize ongoing GABA transmission in a pseudo-competitive fashion. Thus, it might be anticipated that these compounds would be proconvulsant. As mentioned above, MCC is a compound of this type [24, 36, 39, 55]. The allosteric model predicts that compounds having a GABA-shift of 1 should display some unique behavioral properties. Since a compound of this type does not distinguish between ground and activated states of

the macromolecular complex, it should have little or no influence on GABA transmission. However, the model does predict that the drug should block both the tranquilizing effects of benzodiazepines and the proconvulsant effects of MCC. Ro15 1788 is an example of a drug that behaves in this manner [24].

From the foregoing argument it follows that drugs which bind specifically to the benzodiazepine receptor and display benzodiazepine-like activity behave as pharmacological agonists since they *stimulate* GABA transmission, whereas compounds that specifically antagonize the effects of benzodiazepines behave as pharmacological antagonists. Figure 6 illustrates this relationship between the intrinsic activity of benzodiazepine receptor ligands and their influence on GABA transmission as manifested by the "GABA-shift". Although some workers have attributed the different pharmacological effects of benzodiazepines to interactions with different receptor subtypes, we find it simpler to rationalize the diverse behavioral effects of the benzodiazepines as a consequence of their influence on the GABA receptor–chloride ionophore complex. Suppose that the nature of the pharmacological effect caused by benzodiazepine receptor ligands is dependent on the magnitude of the chloride flux induced by the ligand. From this postulate, it follows that the kind of behavior elicited by benzodiazepine-like drugs depends on the dose. It is well known that full agonists (benzodiazepines) have antianxiety effects at low doses, and that at higher doses these compounds cause sedation and ataxia [62, 63]. The low dose, antianxiety effect might result from a small increase in chloride flux caused by the full agonist at low receptor occupancy. At a high level of receptor occupancy, the resulting large increase in chloride flux may be manifest behaviorally as sedation and ataxia. We have suggested previously [9, 60] that the selective anxiolytic, anticonvulsant, CL 218,872, owes its pharmacological selectivity to its lower intrinsic activity as compared to benzodiazepines.

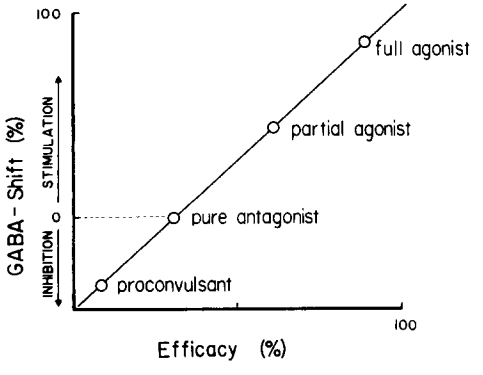

Fig. 6. Relationship between the GABA modulation of binding and the intrinsic activity of benzodiazepine receptor ligands. Ordinate: the "GABA-shift" is given in arbitrary percentage units. A positive value refers to a GABA-induced increase in affinity, whereas a negative value refers to a decrease in affinity. Abscissa: efficacy is given in arbitrary percentage units and is a measure of the extent to which a drug either stimulates (high efficacy) or inhibits (low efficacy) GABA transmission.

Thus, even at high doses, drugs that are *partial agonists* might not stimulate GABA transmission to as great a maximum as that required for the behavioral manifestation of sedation and ataxia. Extending this rationale further, it is possible that the anitconvulsant effects of benzodiazepine-like drugs are the result of an intermediate increase in chloride flux. In agreement with this suggestion are the observations that the partial agonist, CL 218,872, has anticonvulsant effects at high doses, and the weak agonist, PK 8165, displays only anticonflict (anxiolytic) effects.

On the basis of the unique spectrum of pharmacological effects of the triazolopyridazine, CL 218,872, it has been suggested that the type I receptors mediate the anxiolytic effects of benzodiazepines while the type II receptors mediate the anticonvulsant and hypnotic effects [17]. As the theory goes, CL 218,872 is thought to be a selective anxiolytic since it has higher affinity for type I receptors than for type II. Gee and Yamamura [64] have raised several arguments against this hypothesis, the most cogent being that CL 218,872 loses its ability to discriminate between type I and type II receptors at physiological temperatures (37°) [65]. Thus, there does not appear to be a compelling reason to accept the postulate that the type I and type II receptors mediate the anxiolytic and sedative effects respectively.

The conversion of the heterogeneous binding properties of the benzodiazepine receptor at 0° into a state of homogeneity at 37° has also been observed in a study of the influence of temperature on the binding properties of PCC [65–67]. At 0°, it was noted that the PCC/[^{3}H]FLU competition curve has a Hill coefficient of 0.7, whereas at 37° the Hill coefficient was approximately 1 (0.93) [66, 67]. This change in the PCC/[^{3}H]FLU competition curve with increase in temperature was associated with a large, selective reduction in the affinity of the type I receptor, suggesting that the activation energy for the kinetics of the binding of PCC to this site is quite high. An explanation for this temperature-dependent binding affinity is that PCC induces a conformational change in the type I receptor when it binds. It may be that the type II receptors are subject to a greater degree of conformational constraint so that there are observed differences in affinity of these two sites when binding is measured at 0°. Lo *et al.* [68] have found that type I receptors can be separated from type II receptors by preferential solubilization of the type II receptor. These data also suggest that there are differences in the microenvironment of the type I and type II receptors which might possibly provide different conformational constraints. Also, it has been demonstrated that the type I and type II receptors in the hippocampus of the rat are differentially regulated by GABA, suggesting that the natures of the coupling of the type I and type II receptors are different [66, 67]. It has been argued previously that the type I and type II receptors might actually represent different conformational states of the same receptor molecule [64].

At the present time, we do not feel there is sufficient information to construct a definitive model to account for the type I and type II receptors. It

may be that there are differences in the way in which these receptor types are associated with GABA receptors and chloride channels. Also, there may be more than just two conformational states of the benzodiazepine receptor that are populated when different ligands bind upon the receptor. Evidence has been obtained suggesting that there are different ground states (antagonistic states) of the muscarinic [4, 69] and alpha adrenergic receptor [70], in addition to the agonist states of the receptor.

The allosteric model for benzodiazepine receptor function is an attempt to explain many of the pharmacological effects of ligands that interact specifically with the recognition site of benzodiazepine receptor. It has important implications in drug design inasmuch as the development of effective anxiolytic agents that lack the side effects of sedation and ataxia is an important therapeutic goal. Partial agonists which retain high affinity for the benzodiazepine receptor are expected to be selective anxiolytic agents. The development of such drugs depends on the existence of a binding domain on both conformations of the receptor (ground and activated) that does not differ too greatly in structure; otherwise, the decline in efficacy would result in a loss of affinity as well. The allosteric model also predicts that the effects of benzodiazepines on GABA transmission should have a ceiling and not grow proportionately with the dose of benzodiazepine. Perhaps this is the explanation for the high therapeutic index of benzodiazepines when these drugs are administered alone. Although the allosteric model in its current form does not explain all of the complex interactions between ligands at the benzodiazepine receptor, it may provide the basis for an accurate model of benzodiazepine receptor function.

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