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MOLECULAR MECHANISMS IN THE RECEPTOR ACTION OF BENZODIAZEPINES[1]

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E. Costa and A. Guidotti

Laboratory of Preclinical Pharmacology, National Institute of Mental Health, Saint Elizabeths Hospital, Washington DC 20032

INTRODUCTION

An action that clearly distinguishes benzodiazepines from other psychotropic agents is their ability to increase the behavioral output that is depressed by response-contingent punishment or conditioned fear (for review see 1, 2). This specific action has been very useful to establish screening procedures for anxiolytics but has created a number of barriers for studies of the mode of action of benzodiazepines at the molecular level. The traditional classification of benzodiazepines as anxiolytics does not take into account important pharmacological effects such as the myorelaxant and anticonvulsant action (3, 4). Therefore it fails to underline that a most relevant behavioral and pharmacological effect of benzodiazepines, which clearly distinguishes them from other psychoactive agents, is the lack of sedative effects for anxiolytic doses (5). In fact, benzodiazepines in doses that cause the specific increase in punished behavior increase rather than reduce motor activity (1, 2, 6).

The appearance of a large group of benzodiazepine analogues that associate a number of diversified behavioral effects (for review see 3, 4, 7–9) to the antianxiety action has prompted an attempt to define the different benzodiazepines according to their mechanism of action rather than operationally. The present review is an account of the progress made in this effort, but a classification of benzodiazepines based on their molecular activity on transmitter mechanisms is not yet available.

BENZODIAZEPINES AS DRUGS THAT FACILITATE GABAERGIC TRANSMISSION

A multiplicity of neurotransmitter systems, including acetylcholine (10, 11), catecholamines (12–18), serotonin (1, 2, 17, 19, 20), γ-aminobutyric acid (GABA) (21, 22), and glycine (23) have been proposed to participate in the anxiolytic, myorelaxant, and anticonvulsant action of the benzodiazepines. However, only recently have the effects of the benzodiazepines in various parts of the CNS (24–27) been explained with a theory that postulates that GABA transmission is a focal point of convergence.

The evidence supporting this GABAergic hypothesis includes (*a*) the specific ability of the benzodiazepines to relieve convulsions associated with a partial inhibition of brain GABAergic transmission (22, 26, 28); (*b*) the specific action of picrotoxin to block the increase in the punished behavior elicited by the benzodiazepines in rats operating in a conflict test situation (2); (*c*) the capability of the benzodiazepines to enhance GABA-mediated segmental presynaptic inhibition (21, 26) or mimic the presynaptic action of GABA on preganglionic nerve terminals (29, 30); (*d*) the agonistic action of the benzodiazepines on postsynaptic GABAergic mechanisms in a number of brain nuclei or in cultured neuronal systems where GABAergic transmission is operative (9, 26, 31–38); and (*e*) the capability of the benzodiazepines to reduce the increase of cerebellar cGMP content generated by a decrease in the GABAergic transmission between Golgi, stellate, and basket cells and respective postsynaptic neurons (22, 24, 39).

In vitro binding studies with ^{3}H-strychnine and benzodiazepines suggested that the inhibitory amino acid transmitter, glycine, could also be involved in the mediation of benzodiazepine action (23). However, no evidence for an in vivo interaction with glycine was found since in electrophysiological studies the inhibitory response to glycine was unaffected by the presence of benzodiazepines (26, 31–37). In addition, the glycinergic hypothesis for the benzodiazepine action can be discounted because the benzodiazepines do not antagonize preferentially the convulsions produced by strychnine (22, 28).

Schmidt and collaborators (40) first reported that diazepam enhanced presynaptic inhibition in cat spinal cord. However, it was only in 1974 that Polc et al (21), studying the antagonism between diazepam and drugs that block GABA receptor function or inhibit the synthesis of GABA, provided unequivocal evidence for a relationship between GABAergic transmission and benzodiazepines in cat spinal cord. The original electrophysiological studies on spinal cord were extended to other synaptic actions of GABA in other brain nuclei of cats or of other animal species (26, 31–38). The unanimous conclusion was that benzodiazepines act similarly to GABA on

all inhibitory mechanisms that are known to be mediated physiologically by GABA. The selective antagonism by benzodiazepines against convulsions induced by a decrease of the GABA content (24, 26, 28) and the mimicry of the effects of GABA-mimetic compounds on cerebellar cGMP content (39, 41) have fostered the concept that most of the neuropharmacological and neurochemical actions of the benzodiazepines may be mediated via a primary action on GABA transmission.

EVIDENCE INDICATING THAT BENZODIAZEPINES ENHANCE GABAERGIC TRANSMISSION BY A POSTJUNCTIONAL MEMBRANE ACTION

The molecular mechanisms that are operative in the mediation of benzodiazepine action on GABAergic transmission have not yet been completely unveiled but it is almost sure that they are located postsynaptically at the GABAergic junctions. Theoretically, benzodiazepines could act (*a*) by increasing GABA release, (*b*) by inhibiting GABA reuptake either in the membrane of axon terminals or in that of glial cells, or (*c*) by facilitating the GABA actions at the postsynaptic receptor site. In this regard, it is important to note that benzodiazepines do not have a direct GABAmimetic effect.

Evidence indicating that benzodiazepines are not direct GABA receptor agonists was provided by electrophysiological studies (34) on Purkinje cells in the cerebellum (34), on neurons in the sensomotory cortex (33), on neurons in the dorsal raphe nucleus (35), and on cultured neurons (36, 37). However, benzodiazepines potentiate GABA effects. In cat spinal cord, benzodiazepines did not hyperpolarize motor neurons or increase the depolarization of the primary afferent when the tissue content of GABA was decreased by thiosemicarbazide or isoniazid (26). Conversely, these effects could be elicited independently from the GABA content by the GABA receptor agonist, muscimol (9). Thus, it is not surprising that when the benzodiazepines are tested in preparations where GABA transmission is not operative, they may even produce actions opposite to those of GABA (42, 43). Moreover, a direct GABA mimetic action of the benzodiazepines has been excluded because the benzodiazepines (up to 10^{-4} M) added to brain synaptic membranes, prepared as recommended to measure affinity binding of GABA to its receptor (44, 45), failed to displace ^{3}H-GABA from high affinity receptor sites (23, 46). Finally, the increase of cerebellar cGMP and the convulsions generated in vivo by a sizable depletion of GABA content were prevented by a treatment with muscimol but not with diazepam (47). Thus, benzodiazepines exert GABA-mimetic actions only when GABAergic transmission is functional; in contrast, GABAmimetic com-

pounds exert GABAmimetic actions even when GABAergic function is completely inhibited.

Several reports indicate that the benzodiazepines given in anxiolytic doses fail to change GABA metabolism or to increase or decrease the GABA content (35–37, 46, 48–51). Finally, it can be concluded that the benzodiazepines do not exert GABAmimetic action as a result of GABA release from storage sites located in nerve terminals. This was tested in vitro using synaptosomes or brain slice preparations (46, 51) and in vivo by comparing the turnover rate of GABA stored in discrete brain nuclei of rats given benzodiazepines or saline (48).

If the benzodiazepines are not GABAmimetic and do not act presynaptically by modifying the synthesis, release, or reuptake of GABA, it must be assumed that they act postsynaptically by facilitating the action of GABA on receptors by a novel mechanism. This mechanism should depend on specific high affinity binding of benzodiazepines to a regulatory site near the GABA receptor. Perhaps, this site, by an allosteric mechanism either increases the affinity of the recognition sites for GABA, or facilitates the coupling between the GABA recognition site and the ionophore. Consistent with this hypothesis is the description of a saturable high affinity binding site for benzodiazepines different from that for GABA (52, 53) and the recent evidence indicating that the affinity binding of GABA to postsynaptic receptors is modulated by an endogenous protein (54–56). Probably the benozdiazepines induce positive cooperative interactions by acting on this regulatory mechanism and thereby facilitating the binding of GABA to its postsynaptic receptors.

This postulate does not imply that the benzodiazepine binding sites are exclusively located in neurons (57–61) since the benzodiazepines must also bind to glial cells (62) if they contain GABA receptors. Perhaps, the affinity may be lower than that measured for neuronal membranes (63, 64). However, the important point is not the affinity for the binding sites but the function of the benzodiazepine receptors in the regulation of the Cl^- fluxes in glial cell membranes if these fluxes are regulated by GABA. Although any known transmitter, including GABA, fails to compete for high affinity binding sites for the benzodiazepines (52, 53), there are a number of endogenous compounds that have been shown to displace competitively the benzodiazepines from their binding sites (56, 65, 66). Some of these compounds have high molecular weight, others have a very low molecular weight; however, the presence of peptidic bonds was shown only for the high molecular weight compounds (56). In different brain areas the location of high affinity binding sites for benzodiazepines shows a profile which, if not identical with, is a least reminiscent of that for the high affinity binding sites for GABA (44, 52, 53, 57, 58, 60, 61, 67).

The similarity in the brain distribution of GABA and benzodiazepine receptors and the discussed analogies in the pharmacological profile of GABAmimetics and benzodiazepines suggested that studies directed to elucidate the mechanisms whereby benzodiazepine binding sites interact with the GABA receptor sites may help to understand at the molecular level the mode of action of these antianxiety drugs. Although benzodiazepine and GABA receptors are two separate entities, they are probably part of a more complex functional unit. For example, we have recently reported that diazepam, nitrazepam, clonazepam, flunitrazepam, and RO 11-3128(+) increase the affinity of GABA for Na-independent GABA receptors located in freshly prepared crude synaptic membrane preparations obtained from rat cerebral cortex (68).

This effect was dose related; since it was not observed using RO 11-3624(–), a biologically inactive benzodiazepine, it was also stereospecific (68). Furthermore, an interaction between GABA and diazepam receptors located in CNS is supported by the observation that GABA and its analogue muscimol can increase significantly the affinity of the benzodiazepine binding sites, both in vitro and in vivo (69, 70). Such changes in affinity might be expected if the GABA receptors were functionally coupled to the benzodiazepine receptors or if the benzodiazepine binding sites were part of a supramolecular functional entity including GABA receptors and ionophores in which an allosteric activator could play a modulatory role for the intermolecular interactions occurring in the membrane lipid bilayer. Consistent with the latter hypothesis, is the isolation and purification (1500-fold) from brain of a thermostable acidic protein of about 15,000 daltons which can interact with CNS receptors for GABA and benzodiazepines, but not with opiate, dopamine, and muscarinic receptors (54–56). Large amounts of this endogenous protein modulator are present in the brain. The highest concentration has been measured in the cortex, cerebellum, and hypothalamus while much smaller concentrations (approximately one tenth to one twentieth) are present in liver, kidney, and spleen (55, 71). This modulator can be released from crude synaptic membrane preparations by freezing, thawing, and treatment with Triton X-100® (0.05%). When the purified protein modulator was added to synaptic membrane preparations treated as described above, to decrease their modulator content, the affinity binding of ^{3}H-diazepam was reduced by approximately 50% (56). Since the number of total ^{3}H-diazepam binding sites was not changed by the addition of the modulator, the interaction between ^{3}H-diazepam and the endogenous modulator was apparently competitive. This apparent competitive interaction cannot be accounted for by the binding of diazepam to the modulator because the modulator-induced shift in the dissociation constant for diazepam can be observed only after preincubation (30 min or longer) of the

synaptic membranes with the endogenous protein modulator prior to ^{3}H-diazepam addition (56). Moreover an identity between the modulator and the benzodiazepine receptor would be unlikely since the benzodiazepine receptor is a thermolabile unit (58) while the protein modulator does not lose activity after heat treatment (95° X 10 min) (55, 56).

The competitive interaction between the endogenous protein modulator and the benzodiazepines for the same recognition site suggests but does not prove that the endogenous thermostable protein has some of the characteristics expected of a ligand for the benzodiazepine receptor. Having in mind the opiate receptor and its endogenous ligands (72, 73) as a model, the next point to investigate was whether the modulator was the specific endogenous ligand for the high affinity benzodiazepine receptor.

The 1500-fold purified protein modulator of benzodiazepine binding failed to change the high affinity binding of naloxone, QNB, dihydroalprenolol, and spiroperidol to synaptic membranes (55, 74). However, this same protein was extremely potent in lowering the affinity of GABA and the benzodiazepine binding to crude synaptic membrane preparations from brain (54–56). When 0.1–0.4 μg of the 500-fold purified endogenous modulator was added at 0°C to cortical synaptic membrane preparations that were depleted of their modulator protein content, the affinity of GABA receptors for GABA was considerably decreased (from 20 nM to approximately 200 nM) (55). The Scatchard plot analysis of the data revealed that the action of the endogenous modulator on high affinity binding of GABA to its receptors is of a noncompetitive type, whereas the decrease in the affinity of the benzodiazepine receptor elicited by the modulator is competitive in nature (54, 56, 74). Therefore, included among a number of working hypotheses was the possibility that the endogenous modulator operates at the membrane level as an endogenous allosteric effector for the recognition site of GABA receptor. According to this view the modulator might change the conformation of the recognition site of GABA receptor (changing its affinity for GABA) indirectly by activating or inhibiting a process coupled to the function of benzodiazepine receptors.

The experimental evidence that substantiates this hypothesis is still meager; however, it seems significant that diazepam (10^{-9}–10^{-6}M) competes in a dose-related fashion with the modulator for the allosteric regulation of the GABA recognition site. A similar apparent competition was obtained between the modulator and RO 11-3128(+), a pharmacologically active benzodiazepine analogue, but not with its inactive stereoisomer [RO-11-3624(–)] (56, 74). A partial support for this hypothesis came from the similarity in the rank order of the potency of the various benzodiazepines as anticonvulsants and as competitors of the allosteric modulator of GABA recognition sites (56, 74, 75).

Of course, several questions still have to be answered before it can be convincingly concluded that these binding experiments are relevant to an understanding of the molecular mechanisms that are operative in the action of benzodiazepines in the whole animal. In vivo experiments using ^{3}H-muscimol are now in progress to establish whether the potentiation of the muscimol action by the benzodiazepines (76) can be explained by their ability to increase ^{3}H-muscimol binding to brain GABA receptors. The validity of such in vivo documentation, of course, depends on the presence of GABA receptors and endogenous protein modulators in the same cell membranes. Direct support for this association came from studies with cultures of neuroblastoma NB_{2a} and C6 glioma cells showing that their membranes contain high affinity binding sites for diazepam, GABA, and a considerable amount of the modulator protein. Similarly to the brain membranes, diazepam also increased the affinity of GABA receptor for GABA in these cell membranes (74, 75).

INDEPENDENT NATURE OF BENZODIAZEPINE RECEPTOR AND CHLORIDE IONOPHORE CHANNEL

It has been established that the response of postsynaptic neurons to GABA relates to the close functional association of two distinct membrane units, the GABA receptor site (44, 46, 77) and the Cl^- ionophore (77–82). When GABA binds to its recognition site the ionophore channel for Cl^- will open up allowing a Cl^- redistribution across the neuronal membranes according to the concentration gradient (77–82). Therefore, it is possible that the increase of GABA postjunctional response induced by the benzodiazepines might involve an allosteric change in the GABA receptor site and an action at the ionophore site or at the coupling mechanisms between the recognition site for GABA and the ionophore.

Electrophysiological, pharmacological, and biochemical studies are available today indicating that the benzodiazepine receptors and the Cl^- ionophore complex are not the same entity. These conclusions are essentially based on the requirement for the action of benzodiazepines of a certain tissue content of GABA (47), and on the specificity of the interaction between the benzodiazepines and GABA suggesting that a direct effect of benzodiazepines on Cl^- conductance mechanisms used by GABA and glycine is unlikely (37). Furthermore, these conclusions are consistent with the recent description of a saturable high affinity binding for picrotoxin (a marker of the Cl^- ionophore site) to brain synaptic membranes which cannot be displaced by the benzodiazepines (83–85).

Picrotoxin is a potent antagonist of the actions of GABA at synapses (78, 80, 81) and also blocks the facilitatory action of the benzodiazepines on GABA synaptic transmission (24, 29). However, it is believed that the inhibition of GABA synapses by picrotoxin does not occur at the level of the GABA receptors (81). Picrotoxin, like diazepam, does not resemble GABA in chemical structure, and it has been reported to be a noncompetitive inhibitor of the GABA-mediated changes in Cl^- conductance (81). Furthermore, picrotoxin, like the benzodiazepines, even at the concentration of 500 μM fails to inhibit the high affinity binding of GABA to specific recognition sites (45). Based on these considerations, it has been suggested (81, 83–85) that picrotoxin inhibits the changes in Cl^- conductance elicited by GABA receptor activation by directly acting at the ionophore site.

Recent experiments with ^{3}H-dihydropicrotoxinin (a potent picrotoxin analogue) revealed that picrotoxin binds to specific receptor sites in brain (83–84). These binding sites appear to be relevant to explain the pharmacological potency of the drug, and their distribution in crayfish muscle and rat brain (84) closely parallels that of GABA binding sites. Thus, a great deal of evidence supports the conclusion that picrotoxin binding sites are related to the ionophore linked to the GABA receptor (83–85). Olsen and his colleagues have postulated that as a consequence of this interaction, picrotoxin might act by closing the GABA-activated ionophore, thereby blocking Cl^- fluxes through the membranes (85). Consequently, studies of the action of benzodiazepines on ^{3}H-dihydropicrotoxinin binding have become the method of choice to determine biochemically whether the benzodiazepines interact with a specific receptor site of the Cl^- ionophore channel. In synaptic membranes, from rat brain, the benzodiazepines in concentrations up to 10^{-5} M failed to displace ^{3}H-dihydropicrotoxinin from its binding site (83) and vice versa, picrotoxin (10^{-3} M) failed to displace ^{3}H-diazepam binding (52, 53). In this respect, the benzodiazepines differ from the barbiturates which, in vivo, facilitate Cl^- conductance and also are antagonized by picrotoxin (86, 87). The barbiturates in μ molar concentration range displaced the ^{3}H-dihydropicrotoxinin from its receptor site (85) and, conversely, failed to displace the ^{3}H-diazepam from its receptor site in concentrations up to 10^{-3} M (52, 53).

Thus these experiments seem to rule out the possibility of an identity between benzodiazepine recognition site and Cl^- ionophore site and suggest that at the membrane level a functional interaction between benzodiazepine receptor, modulator protein, and GABA receptor is more likely to be responsible for the action of benzodiazepine on GABA transmission and on changes of Cl^- conductance. However, modification of coupling between receptor and ionophore as a consequence of the allosteric change in the GABA recognition site cannot be ruled out at the present time.

THE GABA RECEPTOR REGULATOR UNIT: A MODEL FOR THE RECEPTOR ACTION OF BENZODIAZEPINES

The information we have discussed suggests that the benzodiazepines by acting as an allosteric ligand produce some kind of molecular transitional rearrangement in the supramolecular organization that subserves GABA receptor function. Such an intramembrane allosteric interaction elicited by the benzodiazepines can be expected to alter the mechanisms that physiologically modulate the affinity of GABA recognition sites for their ligand. Consequently, the benzodiazepines modulate the rate of Cl^- ion flux through the GABA receptor-linked ionophore. In postsynaptic membranes of GABA synapses, the elementary functional unit that accounts for the regulation of Cl^- fluxes can be defined as "GABA receptor regulator." Our best understanding of this unit is that it includes a GABA recognition site, a benzodiazepine recognition site, the specific protein modulator, and a Cl^- ion channel.

Among the various components of the "GABA receptor regulator" only the modulator protein has been isolated and characterized. Although GABA, benzodiazepine receptors, and the ionophore can be seen as defined biochemical entities (44, 58, 84, 89), their purification and molecular characterization has not yet been achieved. However, it seems plausible to assume that at least in the membrane of some cells, the fluid lipid mosaic contains GABA and benzodiazepine recognition sites coupled to the modulator protein in a supramolecular arrangement that resembles the association between subunits in regulated enzymes (90). Therefore, with reference to the fluid mosaic model of membranes (91, 92) and as an adapted version of the model of Monod et al (93) for the allosteric protein transition, we can surmise that the "GABA receptor regulator" unit may exist under different conformational states depending on the "quantum" and the nature of the ligand available at the receptor site. In the membranes at rest (no ligands present), a large number of "GABA receptor regulator" units will be as depicted in Figure 1*A*. The diazepam receptor and GABA receptor will be in a nonoccupied low affinity state and the functional state of the ionophore will reduce Cl^- conductance thereby maintaining a distribution of this ion against electrochemical gradients.

When sufficient "quanta" of GABA are released, the occupancy of GABA receptors by the agonist will reduce the restriction of Cl^- fluxes by causing intramembrane translocation of the endogenous modulator with consequent coupling of GABA and benzodiazepine receptor molecules (see Figure 1*B*). As a result of this modification, the affinity of benzodiazepine receptor for the ligand will increase. At rest, the GABA receptor is in a

relatively low affinity state (K_D for GABA = 200 nM); hence, a relatively large amount of GABA is required to stimulate the "GABA receptor regulator" unit. This model is in agreement with the observation that large amounts of GABA are required to increase the affinity of the specific recognition sites for benzodiazepines (69). In contrast, when the benzodiazepine receptor is activated (by addition of benzodiazepines or by a hypothetical and yet unidentified endogenous ligand), the endogenous modulator will lose its control on the GABA recognition site (see Figure 1*C*). As a consequence, a great number of "GABA receptor regulator" units will enter in a high affinity state (K_D for GABA = 20 nM) and will become sensitive to low GABA concentrations. It will follow that in the presence of benzodiazepines the release, from presynaptic terminals, of small amounts of GABA from presynaptic terminals that were previously inactive can activate the "GABA receptor regulator" unit and increase the Cl^- conductance of postsynaptic membranes (see Figure 1*D*). This working model for the interaction of dizepam with the "GABA receptor regulator" unit is in many instances highly hypothetical. Identification and characterization of the different components that are part of the model are still lacking.

The molecular mechanism by which the modulator decreases the affinity of GABA recognition sites from 20 nM to 200 nM is also obscure. The endogenous modulator is a very active endogenous inhibitor of membrane-bound protein kinases (74, 94). Assuming that the membrane protein kinases phosphorylate the GABA recognition site, it can be postulated that

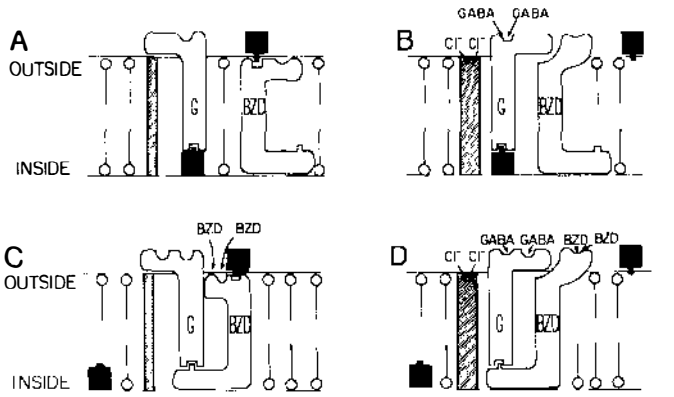

Figure 1 Hypothetical model for the action of benzodiazepines on "GABA receptor regulator unit." A. Resting state. B. GABA receptor stimulation: Cl^- channel opening; C. Benzodiazepine receptor stimulation: GABA receptor affinity increased. D. GABA and benzodiazepine receptor stimulation: Cl^- channel opening; GABA and benzodiazepines receptor affinity increased. G = GABA receptor; BZD = benzodiazepine. The *black square* represents the modulator protein. The *dashed column* represents the Cl^- channel.

phosphorylation unmasks the high affinity recognition sites for GABA; dephosphorylation masks these sites.

Perhaps, the benzodiazepines interact with the modulator protein thereby allowing a certain number of GABA recognition sites to be phosphorylated. The possibility that the modulator of GABA binding alters the kinetic state of GABA receptors by interfering with a phosphorylation process is an attractive hypothesis but needs further experimental support. Finally, the question arises why are the benzodiazepine receptors present; what is the physiological agonist for benzodiazepine receptor site? Perhaps, we can see these receptors as regulatory subunits of a membrane-bound protein kinase, with the possibility that the endogenous ligand is the effector that activates these membrane-bound protein kinase. Indeed, recent reports have appeared which describe small molecular weight endogenous materials (65, 66) (presumably inosine or xanthine) (95) that displace competitively benzodiazepines from their recognition site. It will be of interest to see whether these small molecules are the effectors that physiologically regulate membrane phosphorylation.

CONCLUSIONS

Evidence summarized in this review suggests that the interaction between the benzodiazepines and the allosteric protein modulator of GABA recognition sites may be relevant to explain the in vivo action of the benzodiazepines on GABA transmission. The rank order of potency for a small number of benzodiazepines (74, 75) to compete with the protein modulator is similar to that which causes a relief of anxiety. Whether anxiety expresses a deficiency in the amount of the endogenous protein modulator or in the availability of the proper amount of GABA at the synaptic cleft is not known at the present time, but perhaps can now be investigated.

The use of benzodiazepines as a tool in pharmacological studies has helped to elucidate details of the supramolecular nature of the GABA receptor regulator unit and also has helped to characterize the specificity and efficacy of this group of drugs in the medication of the altered function of GABAergic synapses. Benzodiazepines have no direct GABAmimetic effects. Their only action is that of increasing the efficiency of GABAergic transmission when these synapses are activated. Therefore, it cannot be assumed that GABAmimetic compounds or agents that block GABA metabolism induce the same effects in the whole animal and, in man, as the benzodiazepines do. While the benzodiazepines increase the affinity of GABA receptors for GABA and therefore act with precision providing a fine tuning at the level of synapses where small amounts of the transmitter are released, GABAmimetics or blockers of GABA catabolism will stimu-

late indiscriminately all of the GABA receptors. This implies that their use will bring about a number of side effects; therefore, their therapeutic value will be limited to counteracting a generalized defect of GABA transmission. When needed, a direct GABA receptor agonists with a low intrinsic activity will be preferred for use in therapy.

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