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Biophysical properties and regulation of $GABA_A$ receptor channels

*Robert L. Macdonald**† and Roy E. Twyman**

When GABA binds to the $GABA_A$ receptor, bursts of chloride ion channel openings occur, resulting in membrane hyperpolarization. Barbiturates increase current by increasing mean channel open time, and the convulsant drug picrotoxin decreases current by decreasing mean channel open time. The two drugs bind to allosterically coupled sites on the receptor to regulate channel gating. Benzodiazepines increase and β-carbolines decrease channel opening frequency by binding to the benzodiazepine receptor on $GABA_A$ receptor channels. Neurosteroids increase current by increasing mean channel open time and opening frequency, possibly by interacting with a specific site on the $GABA_A$ receptor. The convulsant drug penicillin reduces current by producing open channel block. The $GABA_A$ receptor subunits contain consensus sequences for phosphorylation by cAMP-dependent kinase, C kinase and tyrosine kinase. The functional consequences of receptor phosphorylation remain unclear. In future studies the use of molecular biological and single channel recording techniques should allow characterization of the properties of $GABA_A$ receptor channels, the role of receptor phosphorylation and the specific mechanisms of actions of regulatory drugs.

Key words: GABA / $GABA_A$ / receptor / barbiturate / benzodiazepine / neurosteroid / convulsant

THE NEUTRAL amino acid γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system, is released from GABA-containing neurons and binds to $GABA_A$ and $GABA_B$ receptors. Its interaction with $GABA_A$ receptors produces a flow of negatively charged chloride ions into neurons, thus producing membrane hyperpolarization. Interaction of GABA with $GABA_B$ receptors produces a flow of positively charged potassium ions out of neurons, also producing membrane hyperpolarization, or reduces the inward flow of positively charged calcium ions. The properties of $GABA_A$ receptor channels are discussed here (for $GABA_B$ receptors; see Bowery *et al*, this issue).

The $GABA_A$ receptor is a macromolecular protein composed of a chloride ion-selective channel with binding sites at least for GABA, picrotoxin, barbiturates and benzodiazepines (ref 36 and other articles in this issue). The $GABA_A$ receptor appears to be composed of combinations of different isoforms of the α, β, γ and δ polypeptide subunits[1,2] (see Tobin, this issue). Cloned receptors composed only of α and β subunits open chloride selective channels when exposed to GABA, are antagonized by picrotoxin and have an increased response in the presence of pentobarbital but lack sensitivity to benzodiazepines;[3,4] the presence of a γ subunit in addition to α and β subunits is necessary for full $GABA_A$ receptor pharmacology.[2] The subunit combinations that are expressed *in vivo* are uncertain. Based upon receptor affinity, presence of co-operativity, and regulation by benzodiazepines, β-carbolines, barbiturates and picrotoxin, it has been suggested that (α1/α3)α5β2γ2 receptors are likely candidates for functionally expressed receptors *in vivo*. (Sigel *et al*[5] describe α5 according to the nomenclature of Pritchett and Seeburg;[6] the same sequence was published earlier and called α4 by Khrestchatisky *et al.*[7])

GABA-mediated inhibition is of major importance in the normal functioning of the nervous system. $GABA_A$ receptors have also been the target of several clinically relevant anticonvulsant drugs and reduction of $GABA_A$-mediated inhibition has been shown to produce seizures. Anticonvulsant barbiturates and benzodiazepines enhance $GABA_A$ receptor function[8,9] but through different allosteric regulatory sites on the $GABA_A$ receptor[10,11] (see also Ticku, and Richards *et al*, this issue). The neurosteroids, which include progesterone and progesterone metabolites, can enhance $GABA_A$ receptor function[12-14] (and see Simmonds, this issue) and have been used in attempts to control seizures in catamenial epilepsy.[15] The mechanisms of action of barbiturates, benzodiazepines and neurosteroids at the $GABA_A$ receptor are here

From the Departments of Neurology and Physiology†, University of Michigan Medical Center, Ann Arbor, MI 48104, USA*

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considered in detail. The convulsant drugs bicuculline, picrotoxin, methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) and penicillin, which reduce GABA-mediated inhibition but each through a different site and mechanism of action on the $GABA_A$ receptor, are also discussed.

Ion selective properties of $GABA_A$ receptor channels

With the application of intracellular recording techniques to the study of central neurons, it was soon discovered that synaptic inhibition was mediated by membrane hyperpolarization. The main inhibitory neurotransmitters were shown to be glycine and GABA. The ion channels activated during inhibitory post synaptic potentials (ipsps) were initially thought to be permeable to chloride ions and to small inorganic anions. This finding led to the suggestion that the receptor ion channels were anion sieves that excluded cations and allowed entry of ions based on their hydrated radii.[16]

Development of the single channel recording technique permitted direct study of these channels on spinal cord neurons in culture.[17,18] When GABA was applied to outside-out patches obtained from mouse spinal cord neurons in cell culture, the $GABA_A$ receptor channel opened and closed rapidly so that relatively square current pulses were recorded (Figure 1.1,2). The currents were reduced in the presence of the $GABA_A$ receptor antagonists bicuculline and picrotoxin (Figure 1.3,4).[19] When outside-out patches were held at −75 mV (inside negative), the current pulses were about 2 pA in amplitude, consistent with a channel conductance of about 27-30 pS. Openings to at least three other current levels were also recorded, suggesting that the $GABA_A$ receptor channel opened to at least four current conducting levels or conductance states (Figure 1.2).[17,18] A 27-30 pS conductance state (Figure 1, double asterisk) was the predominant or main-conductance state, while a conductance state of 17-19 pS occurred less frequently (Figure 1.2, single asterisk).[17,19] Rare openings to 44 and 12 pS conductance states were also recorded. The basis for the multiple conductance states remains unknown

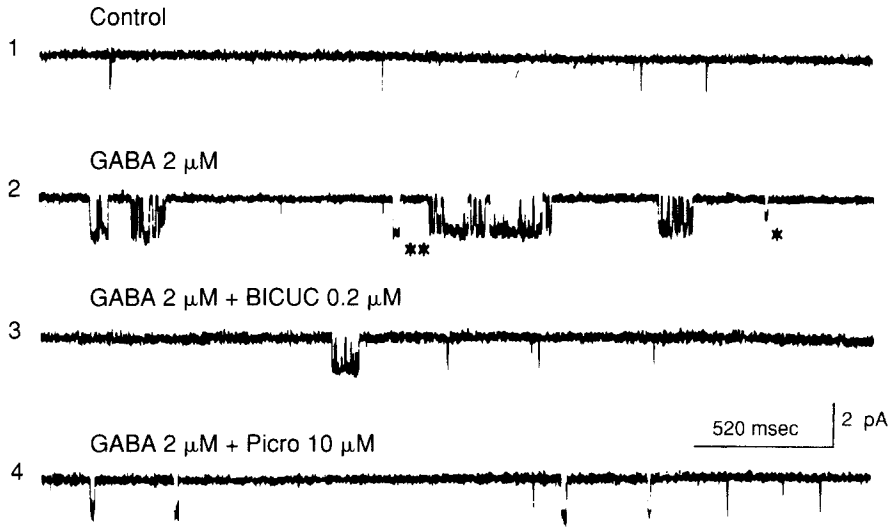

Figure 1. Single channel $GABA_A$ receptor currents recorded from patches of mouse spinal cord neurons using an 'outside-out' patch clamp recording configuration. Membranes were voltage clamped at −75 mV and the chloride equilibrium potential was 0 mV. 1. Before exposing the patch to GABA, rare, brief, spontaneous currents are recorded. Channel openings produce downward deflections of the current recording. 2. GABA (2 μM), applied to the patch using pressure ejection micropipettes, produces an increased frequency of channel openings with a predominant current amplitude corresponding to a main conductance (double asterisk) of about 27-30 picosiemens (pS) and a sub-conductance state (single asterisk) of about 16-19 pS. Openings occur singly or in groups (bursts) of openings. 3. The $GABA_A$ receptor antagonist bicuculline (BICUC) reduces the GABA-evoked current. 4. Picrotoxin (PICRO) also reduces the GABA-evoked current.[19]

but the multiple states may reflect the configuration or combination of different receptor subunits or the distribution of charges within the ion channel.

The chloride channel is composed of hydrophobic membrane spanning regions of each of the receptor subunits (see Tobin, this issue), and the structural and electrical characteristics of the channel pore determine the chloride ion permeability and the conductance of the channel.[1] Based on the permeability sequence for large polyatomic anions, it has been determined that the main conductance state of the $GABA_A$ receptor has an effective pore diameter of 5.6 A.[17] The effective pore diameters of the other conductance states have not yet been determined.

Subunit composition determines the main conductance state: in a Chinese hamster ovary (CHO) cell line stably transfected with cDNAs for $\alpha 1$ and $\beta 1$ subunits of the receptor, single channel recordings have demonstrated that $\alpha 1\beta 1$ $GABA_A$ receptor channels are of small conductance corresponding to the opening of channels to the 19 pS subconductance state.[4] Similarly, in human embryonic kidney cells acutely transfected with cDNAs encoding $\alpha 1$, $\beta 2$ and $\gamma 2$ subunit combinations, $\alpha 1\beta 2$ receptors have a main conductance state of 11 pS; in contrast, $\alpha 1\beta 2\gamma 2$ and $\alpha 1\gamma 2$ receptors had a main conductance state of 30 pS.[20] Which combination of subunits are expressed *in vivo* remains uncertain but it is likely that $GABA_A$ receptor channels with different subunit compositions and different conductance properties are expressed in different neuronal populations.

Gating of the $GABA_A$ receptor ion channel

GABA binds to $GABA_A$ receptors to regulate gating (opening and closing) of the chloride ion channel. GABA concentration response curves are sigmoidal and have Hill numbers of about two suggesting that two molecules of GABA are necessary for full activation of the native receptor channel.[21] It is unclear if the association rates for these two binding sites are the same or if there is cooperative binding. Although the $GABA_A$ receptor opens to four conductance states, current through the main-conductance state is responsible for over 90% of the current through the channel.[19] The single channel gating properties of the main-conductance state of the native $GABA_A$ receptor in murine spinal cord neurons in culture have been characterized:[19,21-23] binding of GABA increases the probability of channel opening and the open channel can close and rapidly re-open to create bursts of openings (Figure 2). With low concentrations of GABA ($<2\,\mu M$) relatively brief, single openings are evoked (Figure 2.2,3), whereas concentrations of $>2\,\mu M$ evoke bursts of long duration openings (Figure 2.4,5).

To explain this complex behavior, the single channel activity of the main conductance state has been modelled using a reaction scheme incorporating two sequential GABA binding sites, three open states and ten closed states (Figure 3).[19,22] In the model, the main conductance-state channel can have at least three open states (O_1, O_2 and O_3) with respective mean open durations of about 1, 3 and 9 ms. Increased concentration of GABA produces increased channel opening and burst frequencies and average open and burst durations without altering the channel conductance (Figure 2).[19] The increased average open and burst durations result not from alterations in dwell times of the open states but from increased frequency of openings of longer open states (O_2 and O_3) and a reduced proportion of openings

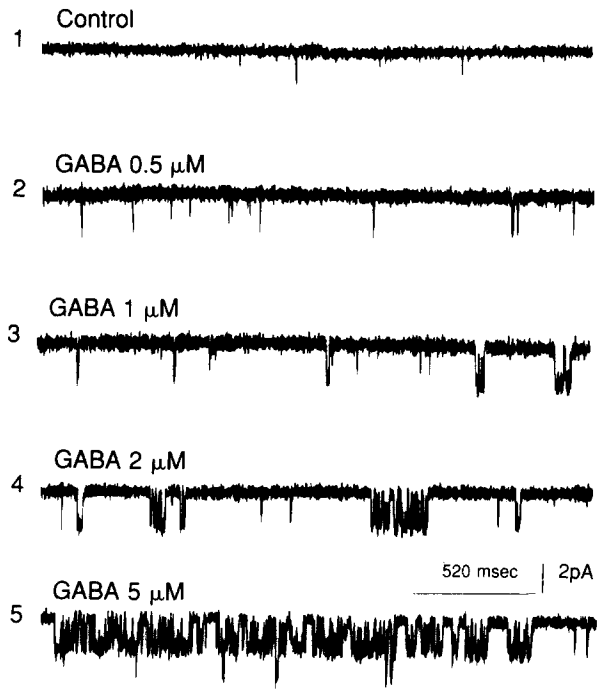

Figure 2. Single channel GABA-evoked currents are concentration-dependent. Recording conditions as for Figure 1. 1. Rare, spontaneous openings are observed before the application of GABA. 2-5. Application of GABA (0.5-5 μM) produces a concentration-dependent increase in opening and burst frequencies (Modified from ref 19).

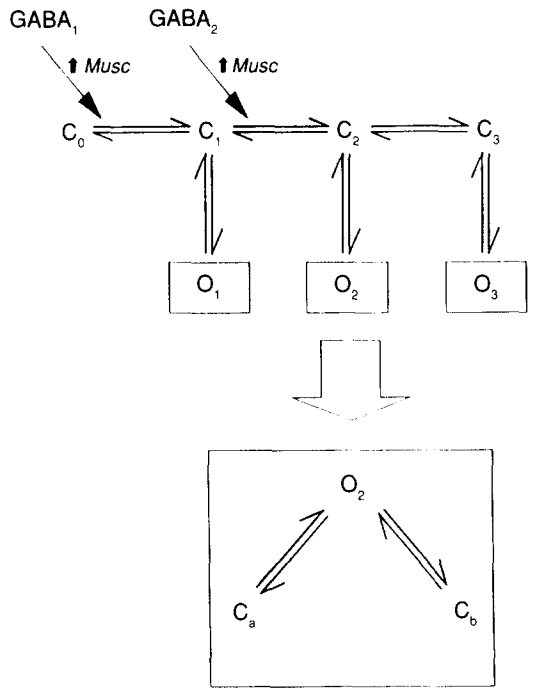

Figure 3. Microscopic reaction scheme for the $GABA_A$ receptor main-conductance state. In this model, GABA binds sequentially to sites $GABA_1$ and $GABA_2$. The channel can exist in multiple open (O_1, O_2, O_3) and closed (C_1, C_2, C_3) states. The open states have respective mean dwell times of 1, 3 and 9 ms. Each open state can produce a burst of openings by oscillating primarily between itself and two adjacent, distal closed states (C_a and C_b) which are shown for O_2 in the expanded box. The brief intraburst closures arising from C_a and C_b have mean dwell times of about 0.2 and 2 ms. The observed mean number of openings per burst are 1, 2 and 4 for bursts derived primarily from open states O_1, O_2 and O_3, respectively. The binding of GABA drives the reaction to the right so that an increased concentration of GABA would produce an increased $GABA_A$ receptor current by an increased frequency of openings and an increased proportion of openings derived from the longer open states (O_2 and O_3). Consequently, burst frequency and average burst duration would also be observed to increase with increased concentration. The potent $GABA_A$ receptor agonist muscimol (MUSC) binds to the $GABA_A$ receptor with increased association rates at both GABA binding sites ($GABA_1$ and $GABA_2$).

of the shortest open state (O_1). From the kinetic reaction scheme in Figure 3, it is apparent that increased GABA concentration will produce an increased frequency of openings. At low GABA concentrations, only a single molecule is likely to bind to the receptor and thus primarily O_1 openings (average open time of 1 ms) would occur. As GABA concentration is increased, a second molecule will bind more frequently to the receptor, and thus, an increased fraction of longer O_2 and O_3 openings (average open times of 3 and 9 ms) should occur.

Bursts of openings are generally organized into a series of openings with 1, 3 or 9 ms mean dwell times. The bursts composed primarily of the 1 ms mean dwell time openings generally have the shortest mean burst duration whereas those composed primarily of the 9 ms mean dwell time openings have the longest mean burst duration. Bursts of openings seem to be produced by oscillations of open states with two distal closed states (C_a and C_b, boxed inset Figure 3). Each of the three open states seem to close transiently into two distal closed states with similar dwell times. Entry into the distal closed states produces the brief closures within bursts (Figures 1 and 2) but the basis for them is uncertain: the brief closures do not appear to be due to open channel block of the receptor by GABA molecules, anions in the bathing medium or anionic buffer. They may represent conformational changes of the receptor channel that occlude the channel transiently regardless which open state is open.

GABA agonists increase the $GABA_A$ receptor current, presumably by acting through one or both of the binding sites for GABA on the receptor. Muscimol, a plant alkaloid and a potent $GABA_A$ receptor agonist,[24] has been used in the treatment of animal models of epilepsy[25] can also precipitate seizures as a toxic side effect.[26] Muscimol evokes $GABA_A$ receptor currents with single channel conductances similar to those evoked by GABA;[27] (R.E. Twyman *et al*, submitted). Results from our laboratory indicate that muscimol evokes bursting currents similar to GABA but muscimol at the same concentration as GABA produces greater open and burst frequencies and average open and burst durations (Figure 4), although dwell times for the three open states are similar (about 1, 3 and 9 ms). Kinetic modelling reveals that these differences may be explained by greater association rates for muscimol binding at each of the $GABA_A$ receptor binding sites. From the kinetic reaction scheme, increased association rates at both of the $GABA_A$ receptor binding sites (Figure 3, $GABA_1$ and $GABA_2$) would increase the frequency and duration of longer openings and bursts. The increased association rates provide the basis for the observed greater affinity of muscimol than GABA for the $GABA_A$ receptor. The potent GABAergic action of muscimol may, therefore, be explained simply by its greater association rates for both of the $GABA_A$ receptor binding sites.

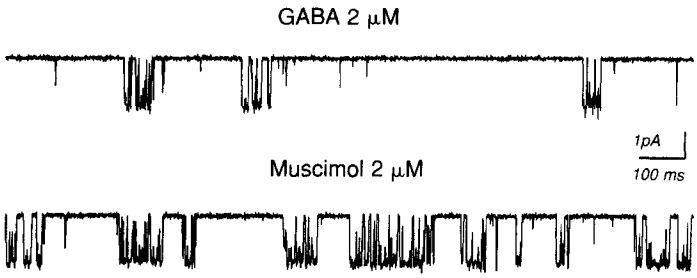

Figure 4. At the same concentrations (2 μM), the $GABA_A$ receptor agonist muscimol evokes more complex currents than GABA. The bursting currents evoked by muscimol (2 μM) are characteristic for GABA evoked currents at a higher GABA concentration (5-10 μM; see Figures 2-5). See the legend to Figure 1 for recording details.

Regulation of the $GABA_A$ receptor

Some anticonvulsant and convulsant drugs can modulate the $GABA_A$ receptor current by regulating the single-channel properties of the receptor. To enhance the current, an agent may increase the channel conductance, increase the channel open and burst frequencies, and/or increase the channel open and burst durations. To reduce the current, an agent may conversely decrease the channel conductance, decrease the channel open and burst frequencies, and/or decrease the channel open and burst durations. The kinetic model of the $GABA_A$ receptor can be used to study the mechanisms of action of anticonvulsant and convulsant drugs that act through the $GABA_A$ receptor.

Bicuculline

Bicuculline reduces $GABA_A$ receptor current by decreasing opening frequency and mean duration (Figure 1.1). Although detailed kinetic studies of the bicuculline effect have not been published, it is likely that it produces a competitive antagonism of $GABA_A$ receptor currents by competing with GABA for binding to the receptor. Whether bicuculline binds to one or both of the GABA binding sites remains uncertain.

Barbiturates and picrotoxin

Phenobarbital has been used to treat patients with epilepsy since 1912. Pentobarbital is also an anticonvulsant drug but its use is limited primarily to the treatment of status epilepticus because it also causes sedation. Barbiturates such as these enhance the $GABA_A$ receptor current by binding to an allosteric regulatory site on the receptor.[28,29] Both pentobarbital and phenobarbital enhance benzodiazepine binding to $GABA_A$ receptors, and pentobarbital, but not phenobarbital, has been shown to increase the affinity of $GABA_A$ binding.[29] Results from fluctuation analysis suggest that phenobarbital and pentobarbital increase the mean channel open duration of $GABA_A$ receptor currents without altering channel conductance.[10,30] Single channel recordings of barbiturate-enhanced single $GABA_A$ receptor currents directly demonstrate that barbiturates increase mean channel open duration but do not alter receptor conductance or opening frequency (Figure 5).[24,27,31,32] On the other hand, analysis of open durations in the presence of clinically relevant free-serum therapeutic concentrations of phenobarbital and pentobarbital reveal that the barbiturates do not alter the dwell times of the three open states of the receptor.[31] Rather, they reduce the proportion of openings with short dwell times (O_1 and O_2) and increase the proportion with the longer dwell times (O_3). Thus, the mean durations of the $GABA_A$ receptor open states are unchanged in the presence of the barbiturates but the average open duration of all openings of the channel is increased. The barbiturates appear to increase primarily the rates of opening of the receptor once GABA is bound (Figure 6). These findings suggest that the barbiturates alter the intrinsic gating of the channel so that openings to state O_3 are increased relative to openings to states O_1 and O_2.

Picrotoxin, a convulsant, non-competitively reduces GABA-evoked currents.[33] Both phenobarbital and pentobarbital can displace picrotoxin binding at the $GABA_A$ receptor although the binding sites for

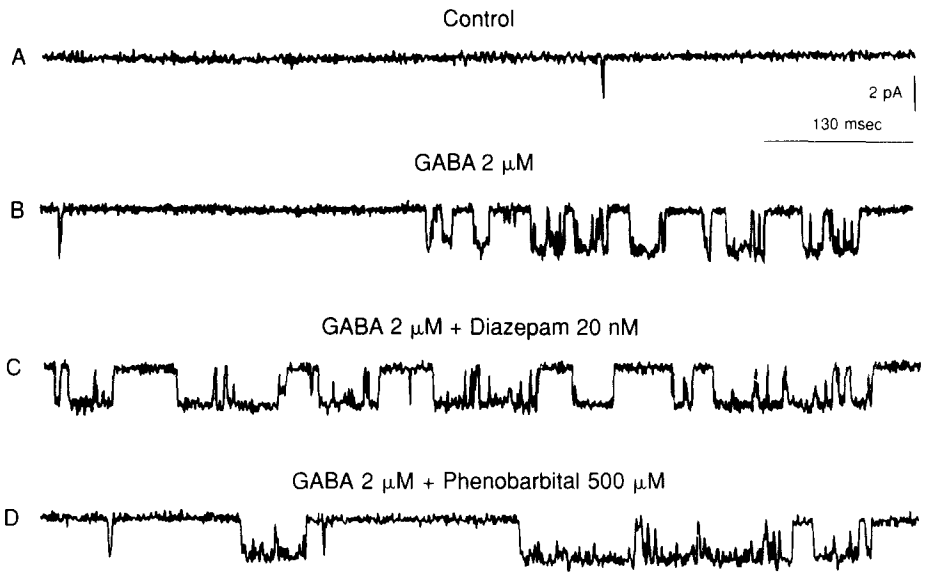

Figure 5. Single $GABA_A$ receptor currents are enhanced by diazepam and phenobarbital. Recording conditions were similar to those described in Figure 1. A. Spontaneous currents in the absence of GABA. B. GABA-evoked bursts of openings. C. GABA-evoked opening and burst frequencies are increased by diazepam. D. Phenobarbital also increases $GABA_A$ receptor currents by increasing the averaged open and burst duration but not the frequency of opening.[11]

these agents are not identical.[29] Thus, the kinetic mechanisms by which picrotoxin reduces GABA-evoked current should be reciprocal to those of the barbiturates. Indeed, single-channel recordings have revealed that picrotoxin reduces GABA-evoked average open duration and burst duration (Figure 1.4).[32] Kinetic analysis of the mechanism for this action suggested that picrotoxin reduces opening transition rates of the bound receptor (Figure 6): entry into the longer O_2 and O_3 open states appears to be reduced more than entry into the briefest O_1 open state. Thus picrotoxin and the barbiturates both seem to act on the same process, gating open the $GABA_A$ receptor channel, but their effect on opening rate constants appears to be opposite—barbiturates favor opening of long lasting open states whereas picrotoxin favors opening of brief open states.

For subunit combinations expressed in *Xenopus* oocytes or in CHO cells, the $\alpha 1\beta 1$ receptor currents were increased by pentobarbital and reduced by picrotoxin.[4,34] Furthermore, the concentration dependence for the effect was the same for receptors with different α and β subunits coexpressed with $\gamma 2$ and with $\beta 2\gamma 2$ alone in *Xenopus* oocytes.[5] These results directly demonstrate that the α and β subunits contain the allosteric regulatory sites for barbiturates and picrotoxin.

Benzodiazepines and β-carbolines

$GABA_A$ receptors have a high affinity binding site for benzodiazepines, and benzodiazepine and $GABA_A$ receptor binding sites have been demonstrated to be allosterically coupled.[29] Evidence has been published suggesting that benzodiazepines may increase the affinity of the receptor for GABA[35] but this conclusion is not universally accepted. Benzodiazepines increase the $GABA_A$ receptor current.[8,9] Results from fluctuation analysis suggest that the benzodiazepine diazepam increases $GABA_A$ receptor current by increasing opening frequency without altering channel conductance or open duration.[10] Single channel recordings have confirmed that benzodiazepines increase receptor opening frequency without altering mean open time or conductance (Figure 5C).[11,36-38] If benzodiazepine enhancement of the $GABA_A$ receptor current were due purely to increased affinity of the receptor for GABA, the single channel kinetic properties should change with increasing concentrations of benzodiazepine in a manner similar to that obtained with increased concentrations of GABA: channel open and burst frequencies and average channel open and burst durations would be expected to increase in the presence of a benzodiazepine. Analysis of single

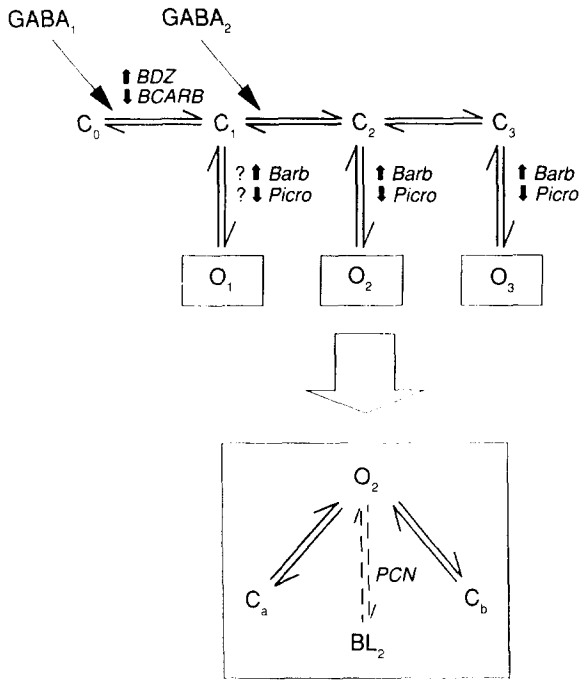

Figure 6. Microscopic reaction scheme for the $GABA_A$ receptor main-conductance state shows binding sites for GABA and proposed sites of action of anticonvulsants and convulsants. See text and the legend for Figure 3 for a discussion of the reaction scheme. To enhance $GABA_A$ receptor current, barbiturates (BARB) appear primarily to increase opening transition rates of bound receptors, thereby prolonging the time spent in open states. The convulsant picrotoxin (PICRO) acts in a reciprocal fashion to the barbiturates. Benzodiazepines (BDZ) modify transition rates or the affinity of the first GABA binding site ($GABA_1$) to increase $GABA_A$ receptor channel opening frequency and thus do not alter average open and burst durations. Convulsant β-carbolines (BCARB) reduce $GABA_A$ receptor currents by a mechanism reciprocally related to anticonvulsant benzodiazepines. The convulsant penicillin (PCN) blocks GABA-evoked openings and introduces a new blocked state distal to each of the three open states (shown as BL_2 for O_2).

channel kinetic properties did not support this expectation:[36] at clinically relevant concentrations of diazepam (< 100 nM) channel open and burst frequencies increase but average open and burst durations are unaltered. These results contrast with the increase in burst duration with little effect on burst frequency seen in the presence of phenobarbital.[11] For diazepam, these results could be explained by an increased affinity of the $GABA_A$ receptor at one but not both of the GABA binding sites (Figure 6, $GABA_1$). More specifically, the increased open and burst frequencies with no change in open and burst durations could be explained by an increased association rate or a decreased dissociation rate only at the first binding site. Alteration of these rates for the second binding site would significantly alter the open and burst durations. Another explanation is that benzodiazepines could reduce the rate of entry into a desensitized state without altering the gating of the bound $GABA_A$ receptor channel.

Reduction of $GABA_A$ receptor currents by an inverse agonist for the benzodiazepine receptor (see Richards *et al*, this issue) is produced by a mechanism opposite to the action of benzodiazepine receptor agonists. Inverse agonists like the convulsant β-carbolines, e.g. DMCM, do not alter $GABA_A$ receptor conductance and average open and burst duration[37] but they do reduce open and burst frequencies. These results suggest that the modulation of $GABA_A$ receptor single channel kinetics by DMCM could be explained by a reduction of the affinity of GABA binding at the first but not both binding sites. (Figure 6, $GABA_1$). Again, an alternative interpretation is that β-carbolines increase the rate of entry into a desensitized state without altering the gating of the bound $GABA_A$ receptor.

$GABA_A$ receptors expressed in *Xenopus* oocytes and CHO cells formed from $\alpha 1\beta 1$ subunits are insensitive to benzodiazepines.[3,4] The basis for this insensitivity was determined when two forms of a third $GABA_A$ receptor subunit, the $\gamma 1$ and $\gamma 2$ subunits, were isolated from a human fetal brain cDNA library.[2] When the $\gamma 2$ subunit was transiently co-expressed with $\alpha 1$ and $\beta 1$ subunits in human embryonic kidney cells, fully functional $GABA_A$ receptors were formed that were sensitive to benzodiazepines, β-carbolines, barbiturates and picrotoxin. Benzodiazepine receptors are heterogeneous with BZ1 and BZ2 receptors having been characterized[39] and the identification of the three specific subunits forming $GABA_A$ receptors led to clarification of the basis for this heterogeneity. Expression of $\alpha 1\beta 1\gamma 2$ $GABA_A$ receptors in human kidney cells produces receptors similar to BZ1 receptors whereas expression of $\alpha 2\beta 1\gamma 2$ and $\alpha 3\beta 1\gamma 2$ $GABA_A$ receptors produces receptors similar to BZ2 receptors.[40] Thus despite the finding that the γ subunit confers benzodiazepine sensitivity to $GABA_A$ receptors, the α subunit appears to be involved in determining the type of benzodiazepine receptor which is expressed. BZ2 receptors are also heterogeneous, being formed from $\alpha 2\beta 1\gamma 2$ or $\alpha 3\beta 1\gamma 2$ subunit combinations. The physiological and pharmacological significance of the differential expression of α subunits remains to be determined.

Neurosteroids

There has been a great deal of interest recently in a variety of steroids and their derivatives that act on $GABA_A$ receptors[12,41,42] (see also Simmonds, this issue). Some endogenous steriods can interact with $GABA_A$ receptors at physiological concentrations and may thus influence central nervous system function during physiological and pathological conditions. It has been speculated that variability of the levels of pregnane metabolites contribute to the development of stress and anxiety and alter seizure susceptibility.[13] Similar to the barbiturates, neurosteroids have been shown to enhance binding to the $GABA_A$ receptor and to allosterically modulate benzodiazepine and TBPS binding to the $GABA_A$ receptor, suggesting that neurosteroids and barbiturates have closely associated binding sites.[14,43,44] Neurosteroids have been shown to potentiate GABA responses in a 'barbiturate-like' fashion.[41,14] Neither neurosteroid nor barbiturate effects are blocked by the benzodiazepine receptor antagonist Ro 15-1788[42] and both steroids[13,41,42] and barbiturates at high concentrations directly activate the $GABA_A$ receptor. The presence of separate neurosteroid and barbiturate binding sites is, however, suggested by results obtained by combining steroids and barbiturates and determining effects on the binding of GABA, TBPS and benzodiazepines.[12,43-45] Direct $GABA_A$ receptor activation by high concentrations of steroids can be further modulated by low concentrations of barbiturate.[13] In contrast to the barbiturates, structurally different neurosteroids can either potentiate or antagonize GABA responses.[46]

Single channel studies of $GABA_A$ receptor modulation by neurosteroids have shown that the conductance of the receptor is unaltered (Figure 7).[13] Prolongation of mean channel open time has been inferred by fluctuation analysis and marked prolongation of single channel burst duration has been reported[13,41] but detailed analysis of single channel kinetics is required to determine the kinetic mechanism of neurosteroid modulation of the receptor.

Penicillin

Penicillin reduces synaptic inhibition and in toxic doses can produce seizures *in vivo*.[47] GABA-evoked responses in the presence of penicillin are reduced in amplitude and prolonged in duration[33] and there is evidence for open-channel blockade of the $GABA_A$ receptor channel by penicillin.[48,49] Open-channel blockers enter open ion channels and physically block current flow, usually completely occluding it when the channel is 'blocked': when the channel is unblocked, the current flow is unaltered. Penicillin reduces average channel open duration and increases average burst duration without altering single-channel conductance (Figure 8).[49] Single channel kinetic analysis reveals that the reduction of open state duration and prolongation of burst duration are consistent with open channel block of the $GABA_A$ receptor. In the $GABA_A$ receptor kinetic scheme, penicillin introduces a distal blocked closed state (BL, Figure 8) for each of the three open states. Penicillin, a negatively charged molecule at physiological pH, must therefore interact with positively charged proteins within the channel intermittently to occlude the flow of chloride ions through the channel.

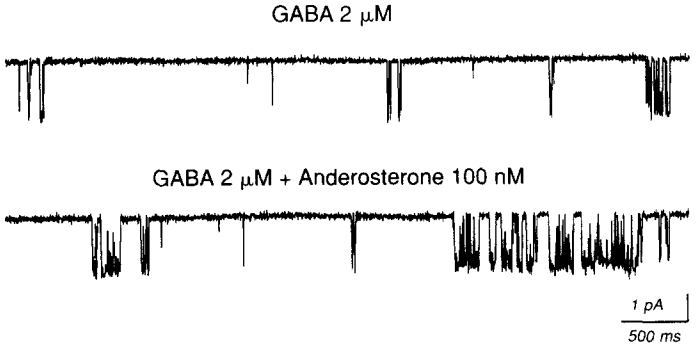

Figure 7. The neurosteroid anderosterone (100 μM) increases $GABA_A$ receptor current by increasing frequency and mean open time of the GABA-evoked openings. See the legend for Figure 1 for recording details.

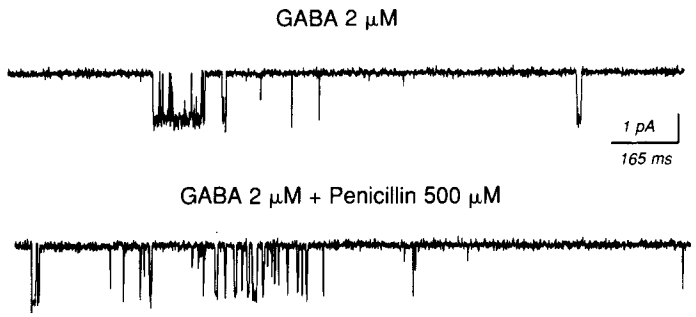

Figure 8. The convulsant drug penicillin (500 μM) decreases $GABA_A$ receptor current by decreasing mean channel open time, increasing burst duration and increasing the number of openings per burst. These alterations in single channel currents suggest that penicillin produces a simple open channel block of the $GABA_A$ receptor channel. See the legend for Figure 1 for recording details.

Phosphorylation of the $GABA_A$ receptor

Cyclic AMP dependent protein kinase A

The β subunit of the $GABA_A$ receptor has been shown to contain a consensus sequence for phosphorylation by cyclic AMP-dependent protein kinase A (PKA).[34] The β subunit of the $GABA_A$ receptor isolated from pig cerebral cortex incorporates ^{32}P in the presence of PKA.[50] Furthermore, PKA phosphorylates purified bovine $GABA_A$ receptor β subunits but detectable phosphorylation of α subunits has also been found.[51]

Because phosphorylation of the nicotinic cholinergic receptor has been demonstrated to enhance desensitization,[52] it was suggested that phosphorylation of the β subunit by PKA might also produce desensitization. Application of lipid soluble cyclic AMP analogues or forskolin, an activator of adenylyl cyclase, to rat hippocampal[53] and chick forebrain neurons in cell culture[54] or rat brain synaptoneurosomes[55] reduced $GABA_A$-evoked current or chloride ion flux. Cyclic AMP, which is not lipid soluble and does not penetrate into cells, also reduced GABA responses when applied to intact neurons, with a potency similar to that of membrane permeable analogs: the membrane permeable analogs of cyclic AMP may not, therefore, depend for their effect on their ability to penetrate cells or to activate PKA.[56]

Recently, the catalytic subunit of PKA (PKA-C) was shown to reduce $GABA_A$ receptor current when applied intracellularly to mouse spinal cord neurons in cell culture.[57] When applied to the inside of outside-out patches obtained from these neurons, PKA-C reduced $GABA_A$ receptor single channel currents by decreasing the frequency of single channel openings without altering their mean open time (Figure 9). This effect was blocked by a specific protein kinase inhibitory peptide, demonstrating its selectivity. Thus, PKA phosphorylates the $GABA_A$ receptor and reduces $GABA_A$ receptor channel opening frequency but the physiological significance of this observation remains uncertain.

Protein kinase C

In addition to being phosphorylated by PKA, the purified $GABA_A$ receptor can be phosphorylated by purified protein kinase C (PKC).[51] Interestingly, PKC also appears to phosphorylate a β subunit but a different one from that phosphorylated by PKA. Furthermore, a splice variant of the γ subunit has been described which contains a consensus sequence for PKC phosphorylation.[58] The significance of phosphorylation of different β and γ subunits by different kinases is unclear but it has been suggested that PKC-mediated phosphorylation of the $GABA_A$ receptor might also produce an alteration in $GABA_A$ receptor responses. Application of phorbol esters, potent PKC activators, reduced $GABA_A$ receptor currents recorded from *Xenopus* oocytes that had been injected with rat brain mRNA.[59] Whether the effect of phorbol esters on the $GABA_A$ receptor current was due to PKC activation or to a direct effect of the phorbol ester on $GABA_A$ receptors remains uncertain. The significance of this potential phosphorylation by PKC awaits experiments in which purified PKC can be applied directly to the interior of cells or to single isolated patches containing $GABA_A$ receptor channels.

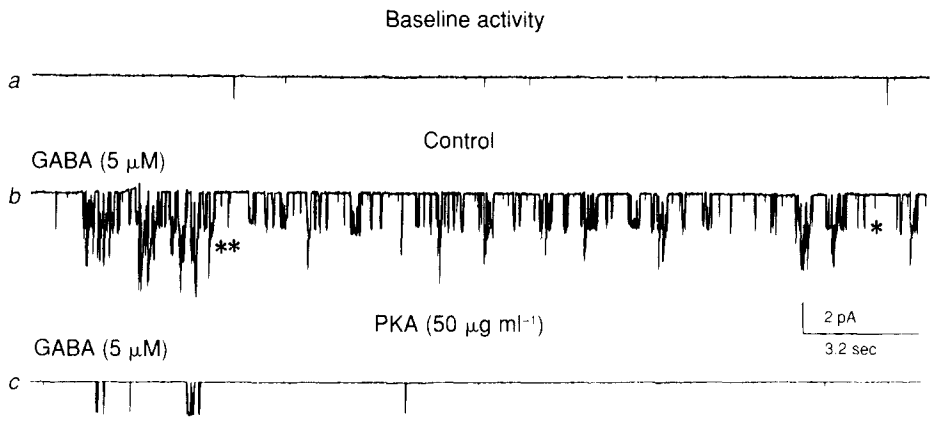

Figure 9. Cyclic AMP dependent protein kinase (PKA) reduces $GABA_A$ receptor single channel currents in excised outside-out patches from mouse spinal cord neurons. Patches were held at −75 mV and recording pipettes contained 2 mM Mg-ATP. *a*. Rare channel openings are present in the absence of GABA. *b*. GABA (5 μM) evokes bursting currents. *c*. Receptor currents are diminished in frequency when PKA is added to the recording pipette.[57]

Outlook

Significant progress has been made toward understanding the biophysical properties of the $GABA_A$ receptor channel but many questions remain unanswered. What combinations of receptor subunits are present in specific central nervous system neurons? Are there developmental changes in receptor subunit composition? Do individual neurons produce one or several types of $GABA_A$ receptors? How is the selection of receptor subtype controlled? What are the physiological and pharmacological properties of $GABA_A$ receptors composed of different subunits?

Cloning of the $GABA_A$ receptor subunits has identified the presence of phosphorylation sites on virtually all of the subunits. Although initial experiments have demonstrated that these subunits can be phosphorylated and that phosphorylation by cyclic AMP dependent protein kinase produces an alteration in the function of $GABA_A$ receptors, the physiological significance of these phosphorylation sites remains uncertain. It is unclear whether phosphorylation by PKC or tyrosine kinase alters $GABA_A$ receptor function. It has not been demonstrated that these receptors are phosphorylated by any of the kinases under physiological conditions or that phosphorylation plays a regulatory role in their function. It is likely that phosphorylation is an important regulatory event but a future challenge is to uncover the role of phosphorylation in the regulation of the properties of these receptors.

Although considerable characterization of the properties of $GABA_A$ receptor channels has been published, the actual physical process of gating of the channel has not been identified and the structural basis for allosteric regulation of these receptors has not been determined. Where are the binding sites for GABA on the receptor? Where on the $GABA_A$ receptor channel is the gate? What is the physical basis for desensitization? How do allosteric regulatory drugs like barbiturates and picrotoxin alter the gating properties of the channel? How do allosteric regulatory drugs like barbiturates and picrotoxin alter the gating properties of the channel? How do benzodiazepines and β-carbolines alter the activation rate of these receptors? Do neurosteroids bind to the same sites as the barbiturates or to unique neurosteroid binding sites? What properties of the gating of the channels are regulated by the neurosteroids? At what site does penicillin bind to block the channels?

These are just a few of the fascinating questions about $GABA_A$ receptor structure and function that remain. With the powerful combination of biophysical and molecular biological techniques that are now available, answers to many of these questions should be forthcoming in the next few years.

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