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New Perspectives in the Functional Role of $GABA_A$ Channel Heterogeneity

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Abstract

γ-Aminobutyric acid A ($GABA_A$) channels responsible for inhibitory synaptic transmission possess a consistent heterogeneity of structure in terms of distinct constitutive subunits. During the past 10 years, considerable progress has been made in understanding the magnitude of this large diversity. Structural requirements for clinically important drugs such as benzodiazepines and barbiturates have been elucidated, and the anatomical distribution in distinct neuronal populations and the developmental profiles of individual subunits have been elucidated with various techniques. However, the relevance of subunit heterogeneity to synaptic transmission is still largely lacking. Recently, substantial progress has been achieved in understanding the crucial role of desensitization as a molecular determinant in defining the duration and frequency responses of inhibitory synaptic transmission. This development, together with a combination of different experimental approaches, including patch-clamp recordings and ultrafast agonist applications in brain slices and mammalian cells expressing recombinant $GABA_A$ receptor, has begun to shed light on a possible role for subunit composition of synaptic receptors in shaping the physiological characteristics of synaptic transmission. Nowhere else in the central nervous system is the anatomical and developmental profile of GABA receptor heterogeneity as well understood as it is in the cerebellum. This review summarizes advances in the understanding of functional correlates to subunit heterogeneity in the cerebellum relevant for inhibitory synaptic function.

Index Entries: GABA; ion channels; benzodiazepines; transfection; receptor subunits; allosteric modulators; $GABA_A$ receptor; desensitization; IPSC; patch-clamp.

Heterogeneity of $GABA_A$ Channels

γ-Aminobutyric acid A ($GABA_A$) channels are responsible for inhibitory synaptic transmission in most neurons in the central nervous system (CNS) (*1*). Membrane hyperpolarization takes place during GABA-ergic synaptic transmission as a result of the fast activation of postsynaptic Cl^- channels by a rapid nonequilibrium exposure of $GABA_A$ receptors to a high

agonist concentration (*2–5*). The $GABA_A$ receptor protein was purified to homogeneity from bovine cerebral cortex by benzodiazepine-affinity chromatography (*6*). Molecular cloning studies have since demonstrated the heterogeneity of these receptor channel complexes, with distinct subunits belonging to different classes. Eighteen different subunits encode genes (α1–6, β1–4, γ1–3, δ, ε, ρ1–2, π) that have been identified from the mammalian brain (*3,4,7–10*). Although we still do not know the precise quaternary structure of these receptors, high-resolution electron microscopic image analysis indicates that they likely consist of a pentameric assembly of distinct subunits (*11*), in spite of reports of the formation of $GABA_A$ channels assembled with a β subunit only (*12*). A major step forward in our understanding of molecular properties of $GABA_A$ channels was the expression of recombinant $GABA_A$ receptors in the cell line HEK293 (human kidney tumor, American type culture collection [ATCC]), which is endogenously devoid of GABA receptors (*12*). Results obtained by cDNA cotransfection (*13*) of HEK293 cells with expression vectors containing the human cytomegalovirus (CMV) promoter-enhancer show that the recombinant $GABA_A$ receptor channels formed show functional and pharmacological properties according to the combinations of distinct subunits (*3,4,8–10*). The six different α subunits have 70–80% amino acid sequence identity among themselves and have been shown to contribute to GABA binding affinity, as well as influencing the action of allosteric modulators such as benzodiazepines and β-carbolines (*3,4,8,9*). By contrast, although an essential requirement for the full expression of functional GABA receptors, the three β subunits appear to be less dominant in regulating the action of the primary transmitter or that of modulators. Three forms of highly related γ subunits have been cloned (*3,4,8–10*). The main functional property related to γ subunit expression is the formation of recombinant $GABA_A$ channels with a main conductance state of 30 pS together with several substates ranging from 10 to 20 pS, as is typically observed with native $GABA_A$ channels (*3*). In the absence of a γ subunit, the channel conductance is maximally 10–20 pS (*14*). Unfortunately, the presence of channel openings at low conductance levels may not be taken as evidence that neuronal membranes may comprise a channel population both with and without γ subunits. However, nonstationary noise analysis performed on inhibitory synaptic currents recorded from cerebellar stellate neurons that most probably express only α1β2/3 and γ2 subunits (*15*) indicate that most openings occur at a 28-pS conductance level, suggesting the possibility that synaptic $GABA_A$ channels are formed by subunit combinations that include the γ2 subunit.

A second important property conferred on GABA receptors by the γ subunits is the sensitivity to positive and negative allosteric modulators such as benzodiazepines and β-carbolines (*3,4,8–10*). This property is a characteristic of all three species of γ subunit; however, it is only with the γ2 and γ3 subunits that the pharmacological profile of benzodiazepine action on most neuronal receptors is reproduced. By contrast, the benzodiazepine potentiation mediated by the γ1 subunit is greatly reduced and betacarboline action is reversed (*8,9*). The δ and ε subunits seem to differ to some extent from the other subunits described thus far. First, their mRNA and subunit leves are more reduced. Second, they are differentially expressed in brain areas in an almost complementary fashion. Third, their assembly into functional complexes imparts a regulation of GABA receptors by neurosteroids and anesthetics (*16*). The neurosteroid modulation of $GABA_A$ receptor channels (*17*) adds to the distinct pharmacological characteristics of $GABA_A$ receptors containing δ subunits, which impacts on the EC_{50} values of GABA dose response, on allosteric modulation by benzodiazepines and barbiturates, and on lanthanum-induced potentiation (*18–21*). The finding that δ subunit cDNA cotransfection decreases neurosteroid potentiation without affecting direct activation of GABA-gated currents (*17*) suggests a distinct site of action for neurosteroid

modulation and a direct activation of $GABA_A$ channel (*22*).

An important clue related to the composition of native receptors comes from studies of δ subunit expression in transgenic α6 subunit knockout mice (*23*). The lack of expression of δ subunit in the cerebellum in these mice indicates that these two subunits are highly likely to be part of a specific cerebellar receptor subtype, as previously proposed with immunoprecipitation studies (*24–27*). Among the pharmacological tools shown to exert a selective antagonism on $GABA_A$ receptor subtypes, lanthanum and furosemide are of considerable interest to this review because they are selective for those subtypes present in the cerebellum (*18,20,26–28*). Lanthanum, a trivalent rare earth metal, potentiates $GABA_A$ receptor activity in dorsal root ganglion neurons (*29*). Lanthanum modulation is distinct for recombinant $GABA_A$ receptor channels comprising the α6 and/or the δ subunit (*18,20*). At the other end, furosemide, a well-known diuretic, is a selective antagonist of $GABA_A$ receptors containing α6 subunits (*28*). These tools are providing a better understanding of the relative role of α6 and δ subunits in shaping inhibitory synaptic transmission in the cerebellum.

The Cerebellum as a Model to Study Functional Roles of $GABA_A$ Channel Heterogeneity

Several reports have demonstrated that $GABA_A$ receptor subunit mRNAs are differentially expressed in distinct cell populations in various anatomical CNS structures, and at different developmental stages (*30–32*). Nowhere else in the CNS is the anatomical distribution and the developmental profile of GABA receptor heterogeneity so well understood as it is in the cerebellum (*27*). *In situ* hybridization studies (*31*) and immunocytochemical techniques (*33,34*) have shown the selective localization of α6 subunits as well as δ subunits in the cerebellar granule, but not in Purkinje cells. Furthermore, immunocytochemical studies indicate that both α1 and α6 subunits are present at synapses innervated by type II Golgi cell terminals in granule neuron dendrites (*35–37*). In the cerebellum, the α1 subunit mRNA is found very early postnatally, whereas the α6 and δ subunit mRNAs could only be detected after postnatal 6 (P6) and P12, respectively (*32*). At later stages, a parallel increase between α1 and α6 subunit mRNAs has been reported between P14 and P21, well after cerebellar granule cell migration, with a peak for both subunits at P21 (*38–39*). The mRNAs for these subunits do not differ between P21, as compared with the adult cerebellum, where the abundance for the α1 message is double that of the α6 mRNA (*38,39*). The δ subunit has been shown to be present predominantly in granule neurons of cerebellum and hippocampus (*31,40*), where it colocalizes with α1, α6, β2/3, and γ2 subunits in the cerebellum and with the α1, α4, β2/3, and γ2L subunits in hippocampus (*31,32*). However, a study demonstrated the transient expression of δ subunits in Purkinje cells of developing rats (*41*). Single-cell reverse transcription-polymeras chain reaction (RT-PCR) studies indicate that although the mRNA for the δ subunit is found in a limited number of cultured granule neurons, its presence in specific cells correlates well with a decreased sensitivity of $GABA_A$ receptors to neurosteroid modulation (*17*). The reduced sensitivity of native $GABA_A$ receptors to neurosteroids during granule neuron development in culture parallels, at least in part, the developmental increase in δ subunit mRNA levels. However, recent anatomical data show that the expression of the δ subunit in granule neurons is limited to extrasynaptic areas (*42*), suggesting that this subunit does not participate in the formation of synaptic receptors in cerebellar granule neurons.

Determinants of Inhibitory Synaptic Currents

At inhibitory synapses, the activation of $GABA_A$ receptors generates inhibitory postsynaptic currents (IPSCs) leading to hyperpo-

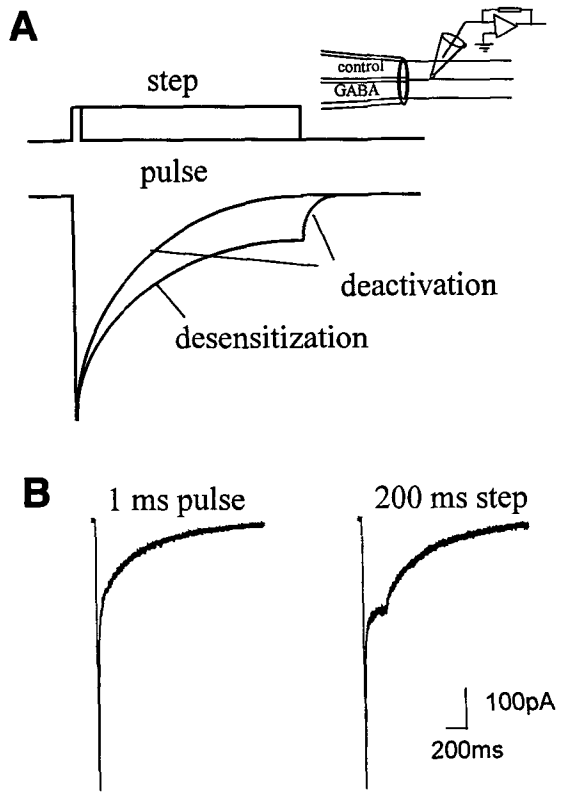

Fig. 1. **(A)** Superimposed representative currents recorded from rapid agonist applications performed with the a piezoelectric translator-based application system that allows one to rapidly apply an agonist (GABA) to an excised patch or cell for desired time intervals while recording currents as shown in the inset in the upper right corner. Continuous flow of solution from a double-barreled glass pipet is maintained throughout the experiment. Crucial to this device is the capability to achieve a solution switch with onset and offset typically <1 ms. (A) The top traces illustrate two distinct experimental protocols. In the first protocol (Pulse), the agonist applied for a short time, as indicated, produces a large sudden activation of the channel in the patch. The declining phase of this current, termed *deactivation,* clearly outlasts the application pulse and is caused by the intrinsic kinetic properties of the channels, rather than the off rate of diffusion of the agonist. In the second protocol, longer agonist applications (Steps) also produce declining currents. In this case, however, the time-dependent current reduction is caused by accumulation of channels in nonconducing (desensitized) states termed *desensitization.* The kinetics of this phase relates to the intrinsic probability of entry and exit from this state. At the end of the step application, the remaining channels that are not desensitized, simply deactivate. **(B)** Current recordings from a nucleated patch excised from a cerebellar granule neurons in a slice from a P14 rat. Currents were recorded upon applications of 1 m*M* GABA for 1 ms *(left)* or 200 ms *(right).* Intracellular patch pipet solution contained CsCl as main salt.

larization of the postsynaptic neuronal cells preventing them from firing action potentials. The duration of the action of GABA at the inhibitory synapse is determined by the time-course of the IPSC. It has been shown by many studies that the IPSC has a rapid rise time and decays with one or two time constants (*43–55*). Mechanisms responsible for multiphasic decay have only recently begun to be understood. Ultrarapid agonist application allows one to avoid bias in measuring current responses produced by the fast desensitization of $GABA_A$ receptors (*5*). This makes it possible to realistically assess macroscopic desensitization (the decline of the GABA response in the continued presence of the agonist) (Fig. 1) and deactivation (the relaxation that takes place after immediate removal of the agonist) (Fig. 1). It is also possible to assess the true EC_{50} of GABA dose responses that are likely to be much higher than previously thought (*52*). Rapid agonist application to excised patches mimics vesicular neurotransmitter release at postsynaptic receptors (*56*). In a way, this methodology allows one to isolate the "pure" postsynaptic receptor contribution to synaptic transmission, bypassing the various mechanisms that control synaptic release. However, an obvious limitation to this technique is that the receptors studied are not necessarily localized at postsynaptic sites. These are likely to be the reasons that currents evoked by a brief GABA application (GABA-activated currents) to outside-out patches have remarkable similarity to IPSCs, but they also differ in several aspects as discussed below in greater detail.

Table 1
Deactivation Kinetic of Cerebellar $GaBa_A$ Receptors

Cell type	Synaptic age (days)	T_{fast}	T_{slow}	%F	Excised Patch T_{fast}	Excised Patch T_{slow}	Excised Patch %F	References
Purkinje neurons	14–18	7.9 ± 1.9	—	100	5.1 ± 2.3	95 ± 36	90 ± 7	*46*
	9–22	8.3 ± 4.3	—	100	—	—	—	*61*
	Adult	7.5 ± 2.4	—	100	—	—	—	*66*
Stellate cells	15–23	9.1 ± 0.7	39 ± 3.4	62 ± 0.04	—	—	—	*62*
Granule cells	8–10	9.1 ± 3	50 ± 2	41 ± 5	7.1 ± 1	85 ± 7	52 ± 4	*50*
	14–18	6.4 ± 0.9	65 ± 19	43 ± 19	4 ± 2	102 ± 48	68 ± 8	*46*
	20–24	6.6 ± 1	43 ± 4	73 ± 4	6.7 ± 1	87 ± 4	58 ± 1	*50*
	Cultured	6.8 ± 0.9	25.6 ± 5.7	50 ± 11	42 ± 4	260 ± 23	73 ± 2	*53*

Comparison of the kinetic properties of IPSC and GABA-activated currents in excised patch from cerebellar neurons. Currents in patches were activated by short GABA pulses (<5-ms duration). The decay time of these currents was fitted with a double exponential curve. T_{fast} and T_{slow} (in ms) are the two time constants of the exponential fitting and %F is the relative fractional contribution to the total peak amplitude of the fast decay component. Data are expressed as mean ± SEM.

Studies of GABA-activated currents in cultured neurons have suggested that the mechanisms underlying the exponential decay components of the IPSC are in part related to the entry and subsequent exit from a desensitized conformational state during which the channel is closed. This desensitized state effectively extends the duration of IPSCs allowing the occurrence of repetitive opening bursts by maintaining the agonist bound (*5,48*). Although kinetic properties of the decay of GABA-activated currents may relate to posttranslational modifications (*49*), kinetic differences are also caused by the expression of different $GABA_A$ receptor isoforms, as discussed later. In fact, it has been shown that, although complex biexponential kinetics could be observed with relatively homogeneous receptor populations, distinct kinetic properties of GABA-activated currents were observed in cells transfected with specific combinations of $GABA_A$ receptor subunits (*57–59*). The following section discusses the importance of subunit composition for setting the properties of cerebellar IPSCs.

Differences in Cerebellar IPSCs

Several reports have characterized IPSCs in neurons in rat cerebellar slices (*15,45,46,51,60–64*) or in primary cultures of cerebellar neurons (*53,65*). Table 1 reports the values of the fast and slow time constants of exponential curves describing the IPSC decays measured in these studies and the relative contributions to the peak amplitude of the responses. Table 1 also compares the values derived from the analysis of the deactivation of GABA-activated currents in patches excised from these neurons, when investigated. It is evident that the distinct kinetics of these currents relate to the nature of the cell investigated, the developmental age, and the nature of the response, whether either synaptic or nonsynaptic. A developmental change is also evident at some synapses investigated.

As widely speculated, the cerebellum is deeply involved in the learning of motor skills. The inhibitory synapses between Golgi cells and granule cells can suppress the excitation of granule cells by mossy fiber inputs and curtail

the duration of excitation, ultimately modulating Purkinje cells, the sole cerebellar cortical output. At P14, a rat opens its eyes and starts moving around, suggesting an extensive postnatal development of motor control, coordination and learning. At this developmental age, the significant change in the decay time course of the granule cell sIPSCs found (Table 1) may be related to the increased α6 subunit contribution to postsynaptic receptors in cerebellar glomeruli. It has been suggested that $GABA_A$ receptors containing the α6 subunit are functionally involved in cerebellar motor control (*67*). It is possible that these changes in sIPSC kinetics might relate to motor learning. At the same time, however, behavioral analysis of α6 knockout mice (*23,68*) did failed to show any gross abnormalities. Therefore, the role of the changes in GABA-ergic synaptic transmission in cerebellar granule neurons occurring during development awaits further insight.

Is Subunit Composition Involved in Determining IPSC Kinetics in Cerebellar Neurons?

To gain further insight into molecular determinants of IPSC kinetics, it is necessary to perform comparative studies of native with recombinant $GABA_A$ receptors selecting compositions defined as much as possible by the results of immunohistochemical studies. Recently, Gingrich et al. (*58*) confirmed the results of Verdoorn (*57*) on distinct $GABA_A$ receptor kinetic properties related to the presence of the α1 subunit versus the α3 subunit. From the kinetic modeling performed in this study, ligand association and dissociation seem to be responsible for both a lower half-maximal concentration (EC_{50}) observed in dose-response studies as well as faster desensitization with the α1 subunit. By analogy, because an even lower EC_{50} is observed with recombinant receptors containing the α6 subunit, as compared with those containing the α1 subunit (*69*), one would expect faster desensitization with α6β2γ2 subunit-containing receptors than with those containing α1β2γ2 subunits. By contrast, the observation that α6β2γ2 subunit-containing receptors do not desensitize (*50,59*) suggests that this subunit coassembly not only affects the binding rate constants but also alters the entry into desensitized states.

As previously discussed $GABA_A$ receptor subunits expressed in the cerebellum comprise the α1, α6, β2/3, γ2, and δ subunits in different combinations, indicating that in cerebellar granule neurons at least three distinct $GABA_A$ receptor subtypes are likely formed, those being the α1β2/3γ2, α6β2/3γ2, and α6β2/3δ combinations (*19,26*). This distinct subunit expression of $GABA_A$ receptors in the cerebellum possibly results in the heterogeneity of receptor function and pharmacological modulation (*45,46,50,53,70*). Studies with α6 subunit-specific antibodies demonstrate that in some glomeruli in adult rats, GABAergic synapses exclusively contain α1 subunits, whereas at other synapses α1 and α6 subunits colocalize in postsynaptic receptors (*36,37*) At the same time, Tia et al. (*50*) demonstrated that the decreased duration and increased sensitivity to furosemide of sIPSCs occurs in parallel during development. This finding suggests that the increased α6 subunit expression underlies the developmental changes in the IPSC decay observed. This hypothesis is further supported by recombinant receptor studies. In fact, when kinetics and furosemide sensitivity of GABA-activated currents were studied in transfected cells expressing recombinant $GABA_A$ receptors, kinetic properties of currents produced by recombinant receptors were obtained only when coexpression of both α1 and α6 subunits together with β2 and γ2 subunits was performed (*50*).

In a further characterization of GABA-activated currents from transfected cells, a larger contribution of the fast decay component to peak amplitude was measured with the α1α6β2γ2 and the α1β2 subunit combination with respect to the α1β2γ2 subunit combination, and a much slower fast time constant for

the α6β2γ2 subunit combination (*50,59*). Since unique kinetics of furosemide sensitive GABA-activated currents from cells cotransfected with α1 and α6 subunit cDNAs were observed (*50,59*), one may assume that the coassembly of these subunits in $GABA_A$ receptors takes place. It is also appealing to speculate that the fast decaying sIPSCs described in older rats are the result of postsynaptic $GABA_A$ receptors coexpressing α1/α6 subunits.

As previously described, the action of lanthanum is related to the presence of the δ subunit. In contrast to furosemide, lanthanum potentiation of sIPSCs in granule neurons was similar in two age groups: P7–P8 and P18–P20 (*71*). Basically, the native $GABA_A$ receptor in these neurons showed a similar modulation by lanthanum as that observed with recombinant α1β3γ2 receptors. Together with the characteristic slow deactivation kinetics and lanthanum inhibition of α6β3δ and α6β3γ2 receptors (*20*), this indicates that the contribution of the these subtypes to inhibitory synaptic transmission of cerebellar granule neurons is negligible. This result further supports the possibility that α1α6β3γ2 subtypes are formed at cerebellar glomeruli with development. Indeed, anatomical findings showed that the α6 and α1 subunits were colocalized in many GABA-ergic Golgi synapses in granule neurons, demonstrating that both subunits are involved in synaptic transmission at the same synapse (*36,37*). Furthermore, the expression of the δ subunit in granule neurons is limited to extrasynaptic areas (*42*).

An important question regards the molecular basis for the slow component of sIPSCs decay in cerebellar neurons. Studies of GABA-activated currents from recombinant receptors indicate that this is unrelated to the presence of the α6 subunit, because it can be observed with α1β2γ2 recombinant $GABA_A$ receptors (*57,58*), it is insensitive to furosemide inhibition (*50,59*) and it is observed in stellate neurons that express only α1β2γ2 receptors. However, because the decay of GABA-activated currents for α6β2γ2 subunit-containing receptors is slower than that of any recombinant receptor tested, the possibility exists that at least in some granule neurons, slow sIPSC decay may be related to the presence of receptors with this subunit combination (*64*).

Role for Phosphorylation in Controlling Desensitization and Differences Between Patches and IPSCs

Responses from excised patches correspond to somatic membranes that may contain unknown mixtures of synaptic and extrasynaptic receptors, whereas IPSCs are produced by the activation of synaptic receptors. Differences between IPSCs and currents in patches have also been reported in Purkinje neurons (*46*), in cerebellar granule neurons in primary culture (*53*), and in slices (*50*), as well as in hippocampal (*48*) and cortical neurons (*52*). In cerebellar granule neurons, patches from soma contain exclusively non synaptic receptors (*36,37*). This possibly explains the results that the developmental decrease in sIPSC duration does not have a match in GABA-activated currents from nucleated patches and that in rats older than P23 the relative contribution of the fast decay component of sIPSCs is larger than that of GABA-activated currents in patches (*50*). The differences between synaptic and extrasynaptic receptors may possibly be related to the presence of distinct receptor subunits. Indeed, immunocytochemical studies in adult rats indicate some differences in α1 and α6 subunit localization at synapses innervated by type II Golgi cell terminals in granule neuron dendrites versus $GABA_A$ receptors found at extrasynaptic sites (*35–37*). However, the differences in kinetics between sIPSCs in cells expressing α1β2γ2 receptors (*15,63*) and deactivation of GABA-activated currents from recombinant receptors of equal subunit composition (*57–59*) indicate that some factor other than subunit composition is likely at play in determining the differences between synaptic and extrasynaptic $GABA_A$ channels. This is further

supported by the unique kinetic characteristics of IPSCs generated at distinct synaptic boutons in cerebellar stellate cells (*63*) of presumptive identical subunit composition (*15*).

Mellor and Randall (*53*) recently reported that in cerebellar granule cells in primary culture the discrepancy between kinetics of GABA currents in nucleated patches and sIPSCs is even greater than that observed in slices (*50*). At the same time, it appears that the effect of Zn^{2+} is much greater in patches than on IPSCs in these neurons (*53*). Furthermore, the action of lanthanum on the slow decay component is larger for sIPSCs than for responses in nucleated patches (*71*). It is attractive to speculate that these results, combined with the greater action of lanthanum on slow components of synaptic currents, allow us to differentiate between synaptic versus extrasynaptic $GABA_A$ receptors and the possibility that the extent of entry into desensitized states is lower for synaptic receptors.

Recently, the previously demonstrated link between phosphorylation and desensitization of the $GABA_A$ receptor (*3,4,9*) has been consolidated (*49*). IPSC kinetics can be modulated by altering $GABA_A$ receptor deactivation and desensitization through phosphorylation (*49*). In particular, it has been demonstrated that calcineurin, a neuronal Ca^{2+}-dependent protein phosphatase (*72*), can alter the deactivation of GABA currents in patches and decay time by increasing unbinding rate. At the same time, however, macroscopic entry into desensitization, the time-dependent decrease of GABA response upon continuous agonist application, is also increased by phosphorylation (*49*). Thus, differences between the kinetics of synaptic and extrasynaptic receptors in the granule cells may be attributable to changes in receptor phosphorylation and these changes may affect IPSC decay rates during development. However, several lines of evidence may indicate that this is less likely. First, the action of phosphatase inhibitors is equally strong on IPSCs in comparison with currents in patches from hippocampal neurons (*49*). Second, inclusion of ATP-—S in the pipette solution failed to reproduce the developmental decrease of sIPSC duration observed (*50*). Third, the developmental decrease of IPSC duration in the granule neurons of both cerebellum (*50,51*) and hippocampus (*54*) has been more convincingly linked to distinct expression of $GABA_A$ receptor subunits because of the distinct and specific pharmacological properties of synaptic currents taking place during development.

An intriguing possibility is raised by data obtained from the comparison of IPSC and GABA responses in patches of visual cortical neurons. In fact, the experimental condition that best reproduces the kinetics of IPSC are those employing very low concentrations of GABA (<100 μM) (*52*). Indeed, the suggested synaptic GABA concentrations derived from other studies are likely <50 μM (*4*). This would suggest that, perhaps at some synapses, a decrease in neurotransmitter concentration takes place during development. This also implies that the postsynaptic response does not involve postsynaptic receptor saturation. Indeed, Nusser et al. (*43*) demonstrated the existence in cerebellar stellate neurons of postsynaptic sites that are clearly not saturated when GABA is released. This elegant correlative anatomical study proves that availability of postsynaptic receptors determines the degree of saturation of the synaptic response. Therefore, perhaps one can envisage a model in which according to specific conditions, an increase in receptor number accompanied by a decrease in the concentration of transmitter released may explain the developmental decrease in IPSC duration. This possibility awaits further testing.

The important role of desensitization in shaping the kinetics of GABA-mediated synaptic transmission and the regulation of desensitization by phosphorylation have prompted Jones and Westbrook (*49*) to propose that distinct protein kinases may alter inhibitory neurotransmission by selectively altering one or more of the microscopic rate constants regulating the distribution of open and close, bound and unbound, and desensitized and non desensitized states.

The duration of IPSC and frequency-dependent desensitization of synaptic responses are certainly keys to the crucial role of the GABA-ergic systems in physiological and pathological aspects of brain function. It is therefore very appealing to think of the possibility that phosphorylation via distinct pathways may produce a variety in the modulation of GABA-ergic synaptic activity. It is also appealing to speculate that distinct phosphorylation consensi on the various $GABA_A$ receptor subunits may allow an even greater level of complexity in the regulation of GABA-ergic synapses.

Neurosteroid and Benzodiazepine Action on Desensitization

The importance of IPSC duration is clearly demonstrated by the profound cognitive effects of drugs that can prolong this important variable of GABA-ergic transmission. The discovery of the distinct roles of subunit composition and phosphorylation in shaping the intrinsic kinetics of $GABA_A$ receptors together with the finding that the main action of allosteric modulators of this receptor is the alteration of the kinetics of synaptic responses (*53,73–75*) prompt the obvious question of the interaction between all these determinants of GABA-ergic function. Although the structural requirements for allosteric modulation are now well established, as described above, a role for phosphorylation in determining the liability to allosteric modulation is less consolidated. Early work by Gynes and Farb (*76*) clearly showed a decrease in the capability to potentiate GABA responses by neurosteroids and benzodiazepines in relation to regulation of phosphorylation. At the same time, it is quite striking that some of the changes produced by benzodiazepines (*53*) and neurosteroids (*77*) are quite similar, involving changes in unbinding rates as well as entry and exit from desensitized states. Benzodiazepines prolong IPSC duration by acting on unbinding; at the same time however, they increase the macroscopic entry into desensitization (*53*). These two action are probably unrelated, as proposed for the effects of phosphorylation by Jones and Westbrook (*49*).

Steroid anesthetics potentiate inhibitory postsynaptic transmission through the prolongation of deactivation taking place during GABA-ergic transmission (*22,54*). Remarkably, the neurosteroid 3α, 21-dihydroxy-5α-pregnan-20-one (THDOC) increases the slow deactivation time constant and slows down recovery from desensitization, as estimated by paired-pulse GABA applications. THDOC also fails to affect the fast deactivation component and its relative contribution to peak amplitude and fails to alter deactivation of short responses induced by a less potent agonist taurine (*77*). At saturating doses, however, THDOC slows deactivation if taurine responses are long enough to permit consistent desensitization. These data strongly suggest that desensitized states are required for the neurosteroid to modulate GABA responses and that the alteration of kinetics of entry and exit from desensitized states underlies the allosteric modification of $GABA_A$ receptors by neurosteroids.

This suggests that the regulation of desensitization and resensitization could be of crucial importance not only in shaping the IPSCs (*5,49*), but also in the allosteric regulation by neurosteroids and benzodiazepines. Future work will permit distinction of the various roles of different compounds on these mechanisms and will show the interactions between these actions and underlying phosphorylation in relationship to subunit composition.

Importance of Frequency-Dependent Control of Inhibition

Potentiation of inhibitory synaptic transmission by allosteric modulators of $GABA_A$ receptors could be achieved by modulation of the frequency, time course and amplitude of spontaneous and evoked IPSCs (*2,5*). As described above, the prolongation of decay time of postsynaptic responses increases the time course of

cell hyperpolarization, leading to increased inhibition of neuronal firing. Also, as proposed by Jones and Westbrook (*50*), activation of $GABA_A$ receptors during inhibitory synaptic transmission will decrease the membrane resistance and thereby the membrane time and space constants allowing the emphasis of temporal and spatial coincidence of excitatory synaptic inputs. In addition, the speed of entry and recovery from desensitization also produces a tight control of the efficacy of inhibitory synapses during high-frequency activity. On the basis of all these considerations, it is clear that inhibitory synaptic function is critical in the timing of neuronal activity. Oscillatory behaviors of neuronal networks are promptly produced by suppressing inhibitory function. Therefore, it is obviously crucial to understand the drug regulation of GABA receptor desensitization that by suppressing neuronal activity at high frequency can allow synchronization of action potential firing even in the presence of intact inhibitory networks. Therefore, what is more important to the behavioral effects of benzodiazepines: the regulation of desensitization, or the duration of the IPSCs? Furthermore, as proposed by Mellor and Randall (*53*), can the spectrum of behavioral effects of diverse benzodiazepine agonists, from anxiolytic to sedative, from anticonvulsant to anesthetic, be related to their specific effects on deactivation or deactivation of synaptic responses? Clearly, much work is needed to understand drug regulation of these properties for native and recombinant $GABA_A$ receptors.

In this regard, distinct frequency-dependent reduction in peak GABA-activated currents was demonstrated from recombinantly expressed $GABA_A$ receptors composed of subunit commonly found in the cerebellum (*59*). These observations demonstrate that $GABA_A$ receptor structure underlies desensitization and resensitization properties, being crucial in setting the frequency response of cerebellar inhibitory synapses. They also show that the absence of desensitization observed with receptors formed with $\alpha6\beta2\gamma2$ and $\alpha6\beta2$ subunit combinations is still observed with high-frequency applications. However, frequency dependent reduction in peak GABA-activated currents demonstrate that, with the exception of $\alpha6\beta2\gamma2$ and $\alpha6\beta2$ subunit combinations, the time constant of recovery from desensitization did not differ significantly among the subunit combinations relevant to cerebellar function.

Future Directions

Recently great interest has grown for the existence of inhibitory synapses that become devoid of postsynaptic receptors, termed silent synapses. Poisbeau et al. (*78*) demonstrated region-specific silent $GABA_A$ synapses during flurazepam withdrawal in the hippocampus. These results are consistent with the hypothesis that chronic benzodiazepine treatment leads to a reduced number of functional synaptic $GABA_A$ receptors in a region-specific manner. At the same time, Nusser et al. (*15*) demonstrated the existence of discrete populations of postsynaptic sites in cerebellar stellate cells, characterized by variations in receptor numbers. These investigators observed the presence of some sites with a very large number of receptors, whereas others possess just a few. This was not the result of a nonuniform receptor density but just to a larger postsynaptic area containing $GABA_A$ ion channels. This observation raises the intriguing possibility that, perhaps in the opposite manner described in the hippocampus during flurazepam withdrawal, certain conditions in the cerebellum increase the strength of GABA-ergic synapses by altering receptor number. This is reminiscent of the response described subsequent to kindling (*2*).

These findings provide evidence for the induction of silent synapses or an increase in the postsynaptic receptors with different experimental paradigms. It will be of tremendous importance to assess whether differences in the subunit composition of synaptic $GABA_A$ receptors in different regions are related to these events. It is therefore crucial to

obtain an understanding of the mechanisms through which allosteric modulators induce selective up regulation or downregulation of specific GABA receptor subtypes. Given that prolonged administration of anxiolytic, sedative, and anticonvulsant drugs can evoke tolerance and dependence by causing uncoupling of the receptor and its regulatory sites (*79*), it will be important to understand whether endogenous mechanisms that alter the ability of such agents to interact with the $GABA_A$ receptors are distinct for distinct receptor subtypes. Several additional questions will have to be answered. Are actions of benzodiazepines related to control of desensitization and frequency dependence involved? Which subunit(s) play(s) a pivotal role in these events? Are silent GABA synapses induced by the phosphorylation/dephosphorylation of specific subunits? These lines of study will pave the way to understanding the molecular mechanisms relevant for tolerance and dependence, and perhaps for endogenous etiopathological events leading to neuropsychiatric disorders.

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