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$GABA_A$ Receptor Plasticity in Alcohol Withdrawal

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SUMMARY

Chronic intermittent ethanol (CIE) treatment and withdrawal in rats produces behavioral changes modeling human alcohol dependence including increased seizure susceptibility, which are associated with long-lasting changes in inhibitory neurotransmission involving the γ-aminobutyric acid (GABA) type A receptors (GABAR). The propensity for dependence on alcohol involves both brain reward mechanisms and the withdrawal syndrome, leading to increased consumption. The withdrawal syndrome includes hyperactivity and hyperexcitability, increased anxiety, sleep disorders, including tolerance to sedative actions of ethanol and other sleep aids, and increased seizure susceptibility. Using a rat model of alcohol dependence involving CIE administration with multiple episodes of intoxication and withdrawal, we deduced that multiple withdrawals produce a kindling-like phenomenon. We also demonstrated that behavioral changes are induced by one dose of ethanol (5 g/kg, gavage) in rats and result from changes in subunit composition, subcellular location, pharmacology and function of GABARs. Ethanol (EtOH)-sensitive extrasynaptic α4/δ-containing GABAR-mediated tonic inhibitory currents are rapidly down-regulated, followed by a slower down-regulation of benzodiazepine (BZ)-sensitive α1/γ2-mediated inhibitory synaptic currents and increased compensatory α4/γ2 synaptic GABAR currents in parallel with increased sensitivity to low millimolar (mM) concentrations of EtOH. While these changes are transient and normalize in a few days, CIE treatment (>30 doses) makes this remodeling of GABAR persistent. We conclude that GABAR plasticity is essential to development of EtOH dependence including seizure susceptibility, and may provide a possible model of epileptogenesis in mammalian brain.

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INTRODUCTION

Alcohol, the fruit of the vine and the braumeister's ware, is one of the most popular drugs in the world throughout history and one of the most abused. The development of dependence after chronic use of ethanol (EtOH) depends on two parallel effects of the drug on the brain each time it is used, stimulation of the reward pathway, and subsequent triggering of a small but significant withdrawal. There is a rebound hyperexcitability following the initial action of EtOH as a CNS depressant, and triggering of some adaptive process, i.e., molecular changes associated with tolerance.[1,2] Each of these little 'mini-withdrawals' reflects transient plasticity in the brain affecting the balance of excitation and inhibition. The simplest description of the changes could be, for example, the ratio of glutamate and GABA neurotransmitter activities.

Whatever the mechanism, a fairly well supported theory of how repeated use of EtOH leads to a dependent condition is that the chronic repetition of the mini-withdrawals leads to a persistent state of withdrawal (alcohol withdrawal syndrome, AWS) in which the withdrawals become more severe and long-lasting, eventually becoming permanent. In other words, repetition turns a relatively normal brain activity involving plasticity into a pathological condition of uncontrolled hyperactivity. This is reminiscent of the kindling phenomenon in epilepsy research, in which seizures can be triggered by subconvulsant stimuli after they have been repeated over and over;[3,4] eventually seizures can become spontaneous, and once they do can occur for the rest of life. One facet of human alcohol dependence is increased seizure susceptibility, and *delirium tremens* and frank seizures are triggered by withdrawal from EtOH in very heavy abusers.[5] Greater susceptibility and/or severity of seizures is produced by greater periods of EtOH abuse and by previous withdrawals and/or withdrawal seizures. When the number of previous exposures and withdrawal episodes reaches a certain threshold, the severe withdrawal (AWS) becomes persistent, possibly permanent. This led to the conclusion of a kindling-like phenomenon in human EtOH dependence.[6–9] However, a significant reduction in seizure threshold can be measured during the mini-withdrawals experienced in rats after EtOH administration.[10,11] This suggests that the seizure susceptibility is, first, an integral component of withdrawal. Second, the increased severity and persistence of seizure susceptibility is a sign of and critical ingredient of alcohol dependence. Numerous animal models employ this 'kindling-like' regimen of intermittent episodes of EtOH intoxication and withdrawal, termed chronic intermittent ethanol (CIE),[12–16] and we have been studying the CIE rat model for 20 years.[11] Indeed, there are many similarities between the development of drug dependence, especially alcoholism, and the kindling model of epilepsy, as well as with other models and theories of epileptogenesis.

Our research has established that an important aspect of alcohol's acute action on the brain is enhancement of inhibition mediated by GABA and especially $GABA_A$ receptors (GABAR), and that withdrawal includes as a critical component a reduction in GABAR-mediated inhibition. The behavioral changes of AWS can be explained by persistently reduced GABAR-mediated inhibition due to EtOH-induced plasticity of GABAR. When this becomes persistent due to the CIE treatment, this can be termed 'aberrant plasticity'. We mentioned how the receptors for the very important rapid neurotransmitters glutamate, and especially GABA, are liable to aberrant plasticity and in a position to do the most harm. In the case of CIE, the treated individual has all the signs of AWS which is an extreme hyperexcitable condition. Increased seizure susceptibility would seem to be a critical aspect of epileptogenesis, but we do not know what additional factors if any are required to generate actual spontaneous seizures (epilepsy). The CIE rats (or mice) have not been observed to exhibit spontaneous seizures but this has not been studied carefully enough to conclude that there are none.

The ratio of excitation to inhibition is so important that a new concept called *scaling* has gained prominence, in which compensatory changes in excitation or inhibition accompany any perturbation of the other (inhibition or excitation).[17] We provide examples in which the deciding factor for aberrant plasticity is reduced GABAergic inhibitory function, which seems particularly susceptible to derangement. These examples cover several chronic drug models as well as epilepsy (see Discussion below). It is known that application of GABAergic drugs, or even GABA itself, to the mammalian cerebral cortex produces withdrawal signs upon removal,[18] that even an hour

exposure can produce long-lasting focal seizures upon termination, the so-called "GABA withdrawal syndrome",[18–20] and that modified GABAR are found in many types of human and experimental epilepsy[21,22] (cf. Macdonald, this volume). Our hypothesis for alcohol withdrawal is that the extrasynaptic GABAR subtypes containing the δ subunit that are most sensitive to low doses of EtOH[23–26], are the first targets of acute EtOH action and the first to show plasticity in the face of chronic EtOH stimulation. Alcohol is accepted to have a GABA-mimetic effect. However, some important effects of EtOH on GABA-mediated inhibition may be presynaptic.[27] Another important concept is that plasticity most often involves changes in protein trafficking[28,29] rather than gene expression, especially for the early events. This does not necessarily apply to the events leading to persistent alterations in drug dependence or epileptogenesis.

RESULTS

The CIE Model and Relationship to Human Alcoholism

Twenty years ago, Kokka and Olsen set out to establish a rat model of the 'kindling hypothesis' of alcohol dependence in humans[6] and to investigate the possible role of GABA$_A$ receptors.[11] The chronic intermittent ethanol (CIE) regimen with 5–6 g/kg EtOH administered to rats by gavage per day for 60 days was found to reduce the seizure threshold to the GABAergic convulsant drug pentylenetetrazol (PTZ) administered by slow tail vein injection, and this change lasted at least 40 days after EtOH was stopped; importantly, the persistence of the changes ('kindling') was dependent on the intermittent drug administration regimen, since continuous administration of EtOH for several days without withdrawal led to one big withdrawal, possibly including seizures, upon cessation, but no detectable change detected at 2 days or any time thereafter.[11] In other words, the animals rapidly forgot about the EtOH exposure but not the multiple withdrawals. Other workers have demonstrated that the intermittent administration of EtOH including periods of deprivation can increase voluntary consumption (e.g.,[14,30]).

We[31] showed that in CIE, GABAR binding was not much affected throughout the brain but that GABAR function, assessed with a neurochemical assay of GABA-stimulated $^{36}Cl^-$ flux in brain slices, was impaired specifically in hippocampal formation, but not in inferior colliculus, several lobes of cortex, thalamus, striatum, or cerebellum. Using extracellular electrode recording in hippocampal slices in collaboration with Dr. Igor Spielman, we demonstrated a parallel reduction in paired-pulse inhibition[31] that was consistent with the increase in behavioral seizure susceptibility. Veatch & Gonzalez[32] presented similar evidence that intermittent EtOH with multiple withdrawals led to elevated excitability specifically in hippocampus, as detected by EEG. We have further showed small changes in benzodiazepine (BZ) modulation of GABAR radioligand binding accompanied by a significant elevation in the GABAR α4 subunit mRNA assessed by *in situ* hybridization histochemistry; the increase was relatively larger in hippocampus than thalamus, despite higher levels of the subunit in thalamus.[33] This is consistent with elevated BZ-insensitive GABAR and behavioral and cellular tolerance to BZ. Indeed, we were able to show with intracellular sharp electrode recordings in hippocampal slices a reduction in allosteric modulation of GABAR-mediated postsynaptic potentials by BZ and steroids, but not by EtOH. EtOH enhancement of evoked synaptic potentials was, if anything, increased.[34] *In situ* hybridization and RT-PCR revealed several changes in GABAR subunits in CIE rat brain including elevated γ2S in hippocampus, and increased binding of the imidazobenzodiazepine radioligand [^{3}H]Ro15-4513 to diazepam-insensitive sites in cerebellum and forebrain, considered to involve the α6 and α4 subunits, respectively.[35] Similar increases in GABAR α4 subunit and smaller changes in some other subunits were observed by others in rodents treated with chronic EtOH (e.g.,[36,37]). Measurements by most groups did not include significant withdrawal periods.

Using subunit-specific antibodies, we measured GABAR subunits by western blotting in CIE rat hippocampus and demonstrated significant and persistent elevation in the α4 and γ2 subunits with a decrease in α1 and δ, in other words, a net 'subunit switch' of α1 to α4 and δ to γ2. The same subunit changes have been reported for several animal models of temporal lobe epilepsy, as reported in this volume by Houser et al., Brooks-Kayal &

Russek, and Joshi & Kapur, as we noted already in the previous edition of Jasper's[21]. CIE animals were shown to exhibit increased anxiety in the elevated plus maze assay, and behavioral tolerance to the sedative action of EtOH, BZ, and neurosteroids.[38] BZ and steroids showed reduced enhancement of GABAR synaptic and tonic inhibitory currents in hippocampal neurons recorded by patch clamp electrodes in CIE rats; sensitivity to the α4-selective agonists bretazenil and Ro15-4513 was not decreased but possibly increased; the GABA analogue THIP showed reduced activation of tonic currents but increased modulation of mIPSCs.[39] These changes in cellular pharmacology are consistent with the net switch of GABAR subunits from α1 to α4. The mIPSCs showed less inhibitory charge transfer due to the contribution of GABAR α4 subunits to the synaptic current measured in CIE rats, as well as reduced tonic inhibitory currents, consistent with the hyperexcitable state.[38,39] CIE rats also exhibited decreased levels of endogenous neurosteroids in the hippocampus, and impaired spatial learning.[40]

Importantly, changes in EtOH pharmacology were also found. We observed significant enhancement of GABAR-mediated tonic inhibitory currents in CA1 neurons and dentate gyrus granule cells by 50 mM EtOH in untreated rats, threshold effect at 10 mM.[41] This enhancement of GABAR-mediated tonic inhibitory currents by millimolar concentrations of EtOH was observed at the same time by Wei et al.[42] in dentate gyrus granule cells and by Hanchar et al.[24] in cerebellar granule cells. CIE rats showed a decreased amplitude of tonic inhibitory current and a correspondingly decreased enhancement by low concentrations of EtOH. On the other hand, mIPSCs showed little effect of EtOH up to 100 mM in vehicle-treated control rats, but were significantly enhanced by 10–30 mM EtOH in CIE rat hippocampus. We confirmed that in the absence of acute EtOH, reduced charge transfer of both synaptic and extrasynaptic GABAR currents was found in CIE rat hippocampal neurons compared to control neurons, consistent with the AWS state, and its associated increased anxiety and disturbed sleep. Further, immunostaining and electron microscopy showed that the α4 subunit, normally localized to the perisynaptic membrane and not in the synaptic membrane, became observed primarily in the synaptic region. The δ subunit is typically not found in the synaptic membrane but is instead localized perisynaptically in controls and did not change in CIE rats.[41] It seems that much of the altered physiology and pharmacology of hippocampal neurons in CIE can be explained by the changes in GABAR subunit composition observed, and this might also explain much of the behavioral phenotype and contribute to EtOH dependence.

The changes found after CIE treatment did not appear to involve any gross pathology in either brain or liver.[31] Microscopic examination of tissue sections revealed no evident changes in morphology, number, and location of GABA-synthesizing neurons in hippocampus, thalamus, or neocortex.[33] Neuronal cell counts (by the UCLA School of Medicine pathology lab) were normal in the hippocampus and several other regions of CIE rats; no increase in damaged or dying cells or inflammation markers was observed. Unbiased stereological cell counts in the nucleus accumbens of NeuN-stained sections showed a lack of differences between CIE, single-dose EtOH, and vehicle treated animals (Spigelman, Ahmad, & Olsen, unpublished). This is despite evidence that exposure to both a single very high dose of EtOH with blood levels over 300 mg/dl, as experienced in human binge drinking, or to a very high level of cumulative alcohol exposure, as in human chronic alcohol abuse, were able to produce significant neuronal cell death.[43,44] We found no evidence for a significant increase in new-born neurons, or stem-cell death in dentate gyrus of CIE rats vs. normal (Spigelman, Olsen, and Crews, unpublished). Thus, in our hands, high blood levels of EtOH administered by gavage, exceeding 250 mg/dl for several hours, but not exceeding 275 mg/dl,[45] were insufficient or too brief to produce the damage reported by other extreme exposures to alcohol. Nevertheless, CIE treatment is definitely a severe, abnormal stress to the brain.

In order to learn more about the mechanism of GABAR plasticity induced by CIE, we attempted to determine the minimum dose, duration, and frequency of EtOH administration required to produce the changes. We found that a single intoxicating dose of EtOH administered by gavage was able to induce many of the same changes in behavior, GABAR subunit composition and hippocampal neuron pharmacology seen in CIE, but the changes were transient.[45] Thus we showed that within one hour the α4 and δ, but not α1 or γ2 subunits, were reduced at the cell surface, accompanied by loss of EtOH enhancement of tonic inhibitory currents but no change in

synaptic pharmacology. Thus the first target of EtOH action, the extrasynaptic δ subunit-containing GABAR,[25] are the first to respond with plastic changes. After 24 hours but not at 1 hour, one could detect increased cell surface and increased total levels of γ2 and α4 subunits, and decreased α1 subunit, and a tolerance to BZ enhancement of both extrasynaptic and synaptic currents. It appears that these changes are the result, at least in part, of altered gene expression. It is not known if these changes are triggered by the reduced tonic inhibition or even the reduced synaptic inhibition seen at several hours post-EtOH, or if altered protein synthesis may be also be initiated by the EtOH exposure, but requires a longer time to be measurable. Also at 12–24 hours, the animals exhibited a tolerance to BZ-induced loss of righting reflex (LORR), and the synaptic currents became more sensitive to EtOH (as in CIE), but returned to normal within a few days. The δ subunit remained low for 1–2 days and then returned to normal.[45] All the changes require the repetition of the CIE regimen to become more persistent. These EtOH-induced plastic changes in GABAR are summarized in Figure 1. Current studies are examining the precise time course of changes in GABAR subunits and function, demonstrating subunit partnerships using co-immunoprecipitation, and examining association of GABAR with other proteins such as trafficking chaperones and clustering factors, as well as kinases and phosphatases, looking for some causal relationships.

One additional observation made about GABAR plasticity induced by CIE[46] demonstrated a correlation between the degree of tolerance induced for a series of GABAergic sedative-hypnotic drugs to produce LORR, and the degree of tolerance induced for the same drugs to enhance GABAR-mediated tonic inhibitory currents in hippocampal neurons. Since the hippocampal neurons do not mediate LORR, or at least not all of the response, we suggest that EtOH-induced changes in extrasynaptic GABAR in other brain regions may be very relevant to the soporific action of these agents. Likewise, since one of the prevalent and problematic signs of alcoholism is insomnia, and resistance to commercial sleep aids, we suggest that those hypnotics that do not show complete tolerance to LORR and modulation of extrasynaptic GABAR in the CIE rat model might retain some efficacy to assuage the insomnia problems of human alcohol abusers.

In summary, the CIE model and our combination of behavioral, biochemical, and electrophysiological measurements have demonstrated the critical role of GABAR in many of the signs and symptoms of the AWS and thus implicated GABAR in EtOH dependence. Remarkable plasticity of GABAR is induced by acute EtOH and the biochemical mechanisms should be decipherable. The same changes are produced by CIE, but they notably become persistent, lasting up to 120 days post-EtOH (Spigelman, Liang, and Olsen, unpublished). Mechanisms for the change to persistence are under study. Approaches include the extension of the CIE model to the mouse to allow use of genetically engineered animals, such as GABAR subunit knockouts and knockins. Knockdown of GABAR subunits in critical brain regions has been demonstrated in rats to reduce alcohol consumption.[47]

DISCUSSION

The CIE Model of Alcoholism and GABAR

Two conclusions have been presented about the rat CIE model of alcoholism and the mechanistic role of GABAR. First, even a single rather large dose of EtOH can trigger within minutes to hours plastic changes in GABAR subunit composition and function, with behavioral correlates, lasting many hours to days, but transient, not persistent.[45] The model has recently been extended to the mouse and to primary cultured rat hippocampal neurons (Olsen & Spigelman, unpublished). Second, chronic administration of moderately high doses of EtOH involving intermittent episodes of intoxication and withdrawal leads to those same plastic changes in GABAR becoming persistent, reminiscent of the kindling of epileptic seizures.[41]

One might ask if the CIE model more closely follows the regimen of the human alcohol abuser who is continually imbibing or a binge type drinker. Although our model requires blood alcohol levels sufficient to

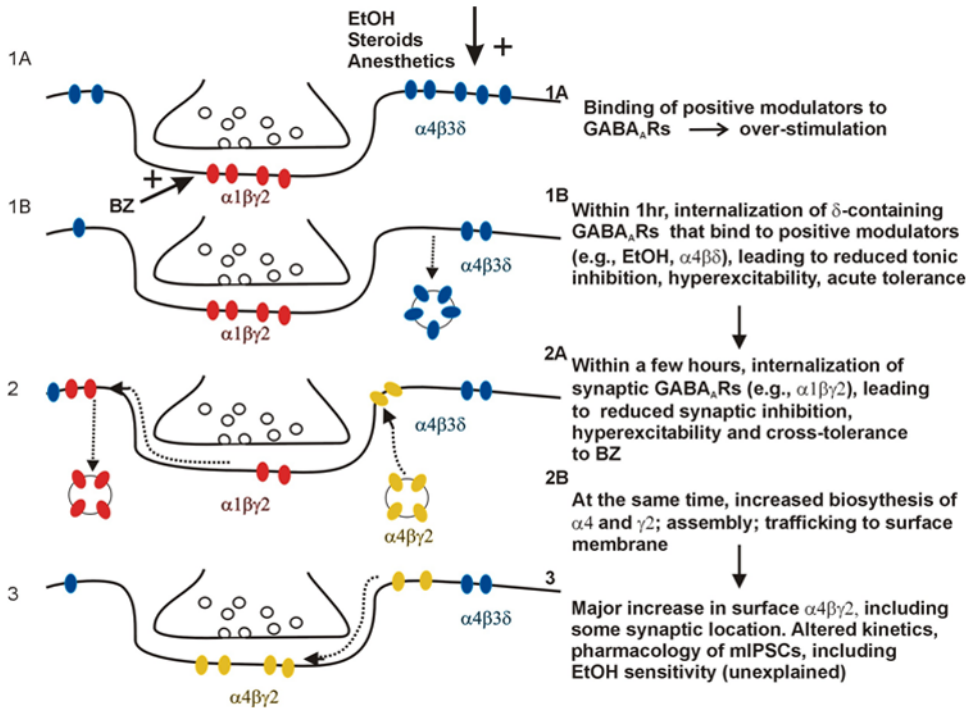

Figure 1. GABAR Plasticity Hypothesis.

produce the mini-withdrawal (ca. 3 hours at ~ 50 mM), the CIE regimen is not exactly like binging since we administer alcohol every day, and we do not reach the blood levels shown in binge studies to be necessary for neuronal toxicity,[44] so we regard the model as more closely approximating the alcohol abuser with sporadic use patterns, which could be fairly frequent, including almost every night but not drinking all day, every day.

We recently have established the minimum dose, frequency, and duration of the regimen needed to produce long-lasting (> 40 days after the last EtOH dose), if not permanent changes in GABAR function and behavior. In the rat this amounts to about 2.5 – 3.0 g/kg per day, once every two days, for at least 15 days (Spigelman & Olsen, unpublished). The major question under study, and of most relevance to the topic of this chapter, is how does the plasticity produced become permanent? This question appears to be highly analogous to the same question raised in epileptogenesis, and only with more understanding of the mechanisms of the change to persistent plasticity can and will cures to the process be developed. We have been examining the period of early plastic changes and later period plasticity induced by CIE in hopes of determining firstly, what is changed, then, what sorts of additional changes occur, and thirdly, how the plasticity becomes aberrant and persistent.

The CIE rats exhibit hyperexcitability in locomotion, rearing, and exploratory behavior. They have a quantitative reduction in seizure threshold to PTZ, increased anxiety, impaired hippocampal spatial memory, perturbed sleep patterns, and tolerance to the soporific actions of EtOH, benzodiazepines, neurosteroids, and several general anesthetics, including most commercial sleep aids. They are not altered in sensorimotor performance. Rodent studies using regimens analogous to CIE lead to increased voluntary consumption, and presumably craving.[14,30] Using patch clamp recording in hippocampal slices from CIE rats, we showed that neurons in CIE rat brain have reduced inhibitory synaptic and extrasynaptic (tonic) currents. They have reduced EtOH enhancement of GABAR-mediated tonic inhibitory currents, but no tolerance for EtOH enhancement of GABAR-mediated inhibitory synaptic currents, and indeed increased sensitivity. Cross-tolerance to BZ and steroids is seen in both synaptic and tonic GABAR currents, and a change in GABAR channel kinetics and pharmacology observed that was consistent with the subunit switch in GABAR observed (replacement of normal subunit subtypes with the α4βγ2 subtype). Synaptic inhibition is reduced in total charge transfer due to the more rapid decay of α4- vs. α1-containing GABAR. The BZ-sensitive α1 subunit and EtOH-sensitive δ subunit are persistently down-

regulated.[41,43] In rats treated acutely with EtOH, these GABAR properties are similarly altered in some other regions, including basolateral amygdala (Spigelman, Liang, & Olsen, unpublished), and probably in ventral tegmentum.[48] In the nucleus accumbens, CIE-induced changes are persistent and pharmacologically similar to the hippocampus. However, single dose EtOH-induced alterations exhibit differences not observed in other brain regions (Spigelman, Liang, & Olsen, unpublished observations).

CIE animals show tolerance to the sedative-hypnotic action of GABAergic drugs, although to varying degree, apparently related to the fraction of action of each drug on the extrasynaptic tonic inhibition.[46] Thus the symptoms, primarily alcoholic insomnia, can still be treated with GABAergic drugs which do not show much tolerance for action on the GABAR-mediated tonic inhibitory currents and in LORR behavior, e.g., propofol, gaboxadol, and barbiturates. Interestingly the anticonvulsant action of EtOH, and especially neurosteroids, and most of the other GABAergic drugs, do not show much tolerance, and the same can be said for the anxiolytic actions.[38,40] The modulation of GABAR-mediated synaptic currents by EtOH does not exhibit tolerance, and in fact the synaptic currents in hippocampal neurons actually become more sensitive to EtOH.[34,41,45] This increased modulation of mIPSCs by EtOH is also seen in the GABAR α4 subunit knockout mouse and might account for the lack of reduction in many EtOH behaviors in these mice.[49,50]

Therefore we asked: what might be the subunit composition of GABARs accounting for this increased EtOH sensitivity of synaptic currents? In CIE we observed increase in α4βγ2 GABAR including movement of the α4 into the postsynaptic membrane. The δ subunit was not elevated, did not accumulate in the synaptic membrane, and the increased EtOH modulation of mIPSCs was also observed in the GABAR δ subunit knockout mouse.[41] The increased sensitivity to EtOH in mIPSCs in the α4 subunit knockout mouse rules out the α4βδ and α4βγ2 for the EtOH-sensitive GABAR pentamer, so we are examining other possibilities. One cannot help suggest that some unknown factor(s) other than subunit composition alone might affect EtOH sensitivity. Perhaps this is related to subcellular location, and/or associated proteins, and/or some protein phosphorylation event(s).

Other Studies on Chronic EtOH and other Drug-Induced GABAR Plasticity

GABAR subunit changes have been reported by others in chronic EtOH-treated animals or cells.[37] Mhatre et al.[51] demonstrated that chronic EtOH treatment in rats produced a hypersensitivity to the behavioral effects of the benzodiazepine alcohol antagonist Ro15-4513, followed by biochemical evidence[36] for an up-regulation of [^{3}H]Ro15-4513 binding to diazepam-insensitive (DZ-IS) sites (shown elsewhere to reflect α4- and α6-containing GABAR). We showed that CIE treatment led to elevated levels of α6 mRNA and protein in cerebellum as well as elevated γ2S mRNA in forebrain[35] and elevated α4 mRNA in hippocampus but not thalamus or cortex.[33] Western blots revealed elevated α4 and γ2 protein as well as reduced α1 and δ polypeptides in CIE hippocampus.[38] In retrospect, the elevated Ro15-4513 binding polypeptide reported in forebrain following chronic EtOH by Mhatre & Ticku[36] was undoubtedly the α4 subunit. Meanwhile the lab of Morrow et al. showed that chronic EtOH led to elevated levels of α4 and reduction of α1 involving phosphorylation-regulated trafficking in some forebrain regions.[52,53] A few other subunits were altered, including especially elevated γ1. Similar subunit changes were observed in the lab of Biggio and colleagues for rats and neurons, with an emphasis on the requirement of several hours of withdrawal for EtOH-induced plastic changes in GABAR subunits and behaviors.[54] What falls out of the summarized literature is that chronic EtOH leads to up-regulation of the GABAR α4 subunit, which appears to be a very 'plastic' subunit, subject to changes in levels under many conditions of stress or over-activity.[21] The α4 subunit is available to replace other α subunits and this leads to tolerance to sedative drugs given chronically, but also an abnormally excitable state. It suggests the possibility that in the epilepsy models there is an overactivity of GABA-mediated inhibition leading to a similar increase in α4 which has some benefit but also produces hyperexcitability. Recent work describes how the GABAR α4 subunit is indeed regulated at the promoter level for gene expression by immediate early genes including heat shock proteins[55,56] and early growth response factors (Egr3)[57,58], which are elevated under various stressful and overstimulation conditions including alcohol exposure[56] and prolonged seizures[57,58]. The

α4 subunit polypeptide can be partnered to γ or δ subunits leading to different pharmacological and functional properties, and thus which of these isoforms is involved is likely very important. We have shown that in fact the α4βδ GABAR are down-regulated and the α4βγ2 GABAR are up-regulated[38,41] and play an important role in AWS and EtOH dependence, and exhibit a profound example of aberrant plasticity in the CIE model.

Interestingly, Hu & Ticku[59] were able to reproduce many of the effects of CIE on rats *in vivo* on cortical cultured neurons *in vitro*, including hypersensitivity to blockade of GABAR function by PTZ. Primary cultured neurons may therefore provide a good model of alcohol dependence allowing more defined studies of mechanism including GABAR plasticity. It is critical to establish that primary cultured neurons can mimic as much as possible of the phenotype of the mature brain neurons and the critical plastic changes induced by EtOH. We have now demonstrated in primary cultured hippocampal neurons that one brief exposure to EtOH produces many changes in GABAR[60] that we found *in vivo* in rats[45].

GABAR changes have been seen in animals or cells treated with chronic BZ.[61–66] In all these examples, one can conclude that over-stimulation of the receptor by any positive modulator or agonist leads to down-regulation of the GABAR subtypes, and only those, that are sensitive to that ligand. In some cases the function (chloride current) is monitored whereas in other cases BZ modulation of GABA site ligand binding is reduced (uncoupling). All of these examples are likely due to a removal of the activated GABAR protein from the cell surface, which, depending on the time of study, can result in reversible return to normal or at longer times in degradation of the receptor protein. The BZ effects are limited to action on the subtypes of GABAR that respond to BZ, namely the γ2-containing isoforms containing α1, α2, α3, or α5[62]. The result is not just a loss of BZ modulation of GABAR function but a silencing of the GABAR function at that site for a certain period of time like hours to days (e.g.,[63]). The loss of cell surface GABAR by endocytosis can be demonstrated. However, basal binding of BZ ligands may persist because the protein in intracellular pools can bind ligand but it is not subject to modulation by GABA in the test tube ("uncoupled") because of the low pH of the endosome environment.[62,64,66] GABAR plasticity is also triggered by chronic[67,68] or acute administration of neurosteroids.[69,70] Chronic administration of steroid as a drug produces CNS depression with a measurable withdrawal[68] that is accompanied by a tolerance of electrophysiologically recorded GABAR currents to steroids but more dramatically to BZ, accompanied by a switch in subunit composition from traditional BZ-sensitive synaptic GABAR (α1, α2, α3, α5) to the BZ-insensitive α4 subunit. There does not appear to be a down-regulation of the highly steroid-sensitive α4βδ GABAR subtypes. The *in vitro* and *in vivo* pharmacological changes as well as the withdrawal behavior are all reduced by preventing the increase in α4 subunit with anti-sense mRNA.[63]

The plasticity appears to be a variant on the usual mechanism of use-dependent down- regulation. More importantly, the neurosteroids are endogenous ligands with *in vivo* mechanisms of homeostasis, including linkage to the endocrine system.[67,68,71,72] It might not be healthy if steroid-sensitive GABAR were down-regulated every time they were modulated for more than a few sec by endogenous modulatory neurosteroids. And what sort of severe over-stimulation by GABA itself is required before down-regulation is triggered?

Indeed the function of GABAR is tightly coupled to the state of neurosteroid activity in the CNS. It appears that high levels of stimulation of the highly sensitive α4βδ type GABAR by neurosteroids leads to plasticity, probably involving similar mechanisms to those mentioned above. GABAR changes are observed in pregnancy and parturition,[72,73] at puberty,[74] and during the estrus cycle,[75] possibly related to premenstrual syndrome. Neurosteroids have even been postulated to mediate at least some of the effects of EtOH occurring at minutes to hours after administration.[76] It is therefore likely that neurosteroids play a role in EtOH-induced plasticity of GABAR observed in AWS and dependence. These are early days in this field but implications for epileptogenesis are obvious (cf. Mody, this volume).

GABAR Plasticity in Epilepsy

GABAR changes similar to that described in CIE have been reported after seizures, status epilepticus, and in other epilepsy models.[21,22,77] Nusser et al.[78] demonstrated that experimental epilepsy leads to an increased number of synaptic GABAR measured by electron microscopy in rat hippocampus. Brooks-Kayal & Russek (this volume)[79] showed that epileptic animals exhibit a switch in GABAR from α1 to α4, and that development of epileptogenesis could be inhibited by preventing the GABAR subunit switch.[80] Banerjee et al.[81] observed a decrease in allosteric modulation by neurosteroids of [^{35}S]TBPS binding to GABAR during absence seizures in rats only in the affected brain cells in thalamus. We interpreted this to reflect a switch in GABAR subunit composition, involving both trafficking and gene expression. This was further supported by Banerjee et al.[82] who demonstrated rapid (within 1–2 hr) changes in GABAR α1 and α4 subunit gene expression in thalamic neurons during absence seizures. Naylor et al.[83] demonstrated that status epilepticus produced a down-regulation in the γ2-containing synaptic GABAR, interpreted as an over-stimulation by massive GABA synaptic release; they also postulated a protection of extrasynaptic GABAR mediating inhibitory tonic currents. Subunit-selective regulation of synaptic GABAR trafficking and localization has been demonstrated by Kapur and colleagues[84–86] (Joshi & Kapur, this volume). The mechanism of status epilepticus-induced internalization of synaptic GABAR has been shown to involve increased dephosphorylaton-regulated binding of GABAR to clathrin for endocytotic removal from the cell surface.[87] Status epilepticus-induced spontaneous seizures were shown to be accompanied by alterations in the synaptic vs. perisynaptic localization of α4βγ2 and α4βδ type GABAR (Houser et al., this volume)[88].

In conclusion, we suggest that the plasticity observed in alcohol withdrawal involving GABAR and especially extrasynaptic α4βδ type GABAR is indicative of the common use of GABAR (and GLUR) plasticity in normal brain function including learning and memory. Further, the mechanisms responsible for GABAR (and GLUR) plasticity have the propensity for aberrant plasticity leading to seizure susceptibility: a model of epileptogenesis? We continue to study this hypothesis. Current studies involve CIE treatment in the mouse and examination of genetically engineered animals, e.g.,[89,90], and in a cultured rat hippocampal neuron model[60]. Finally, recent studies increasingly implicate changes in GABAR-mediated tonic inhibition in epilepsy physiology (see chapters in this volume by Houser; Mody; Brooks-Kayal & Russek; and Joshi & Kapur).

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