REVIEW —Current Perspective—

Contribution of Glutamate Receptors to Benzodiazepine Withdrawal Signs

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ABSTRACT—Recent research has demonstrated that the receptor for glutamate, a major excitatory neurotransmitter, may play an important role in the expression of benzodiazepine withdrawal signs. This proposal is based on various observations. For example, antagonists for N-methyl-D-aspartate (NMDA), non-NMDA and metabotropic glutamate (mGlu) receptors can suppress the behavioral signs of benzodiazepine withdrawal in mice and rats. Furthermore, the NMDA receptor in the cerebrocortical area of diazepam-withdrawn rats is upregulated. Finally, the stimulation of phosphoinositide hydrolysis mediated by mGluR is enhanced in cerebrocortical slices from lorazepam-withdrawn mice. These findings show that the upregulation of signal transduction mediated by glutamate receptors during diazepam withdrawal plays a role in the neuroadaptive response responsible for the expression of diazepam withdrawal signs. Furthermore, ligands for glutamate receptors may be suitable targets for treating benzodiazepine withdrawal signs.

Keywords: Benzodiazepine, Withdrawal sign, Glutamate receptor

Introduction

Since the first benzodiazepine derivative chlordiazepoxide was developed by Roche in 1960, numerous benzodiazepines have been synthesized and prescribed. The potent anxiolytic, anticonvulsant, hypnotic and muscle relaxant effects of benzodiazepines have led to a rapid increase in their use for the treatment of anxiety, tension, insomnia and vague psychosomatic complaints. Compared with traditional anti-anxiety agents such as barbiturates, chloral hydrate, meprobamate, paraldehyde and alcohol, benzodiazepines were thought to be safer and more effective for the long-term treatment of anxiety and insomnia. However, the inappropriate and long-term therapeutic use of benzodiazepines have become especially important in light of the accumulation of data regarding the development of physical dependence (1, 2).

Physical dependence has been defined as a state in which the discontinuation of drug treatment is followed by the expression of a time-limited withdrawal reaction that can be reversed by the resumption of treatment. In general, withdrawal signs are opposite the direct pharmacological effects of the drug. For example, benzodiazepine withdrawal signs in humans have been characterized by anxiety, muscle spasms, seizure, weight loss and even mortality (1, 2). These withdrawal responses have been considered to reflect neuronal hyperexcitability as a result of neuroadaptive responses to chronic treatment with benzodiazepines since benzodiazepines facilitate the actions of γ-aminobutyric acid (GABA), the major fast inhibitory neurotransmitter in the central nervous system (CNS), resulting from binding to a high-affinity site for benzodiazepines on the GABAₐ receptor.

Glutamate is the major fast excitatory neurotransmitter in the CNS and plays a role in many important normal and pathological functions such as epileptic disorders. Glutamate receptors have been classified into several different subtypes. These include N-methyl-D-aspartate (NMDA), non-NMDA and metabotropic glutamate (mGlu) receptors. The NMDA- and non-NMDA-receptor subtypes are ligand-gated ion channels, and mGlu recep-
tor (mGluR) is coupled with a G-protein. Many studies have noted that these glutamate receptors contribute to the physical dependence caused by ethanol, barbiturates and opioids (3), and we and others have recently demonstrated that these glutamate receptors are involved in physical dependence on benzodiazepine. In the present review, to focus on the role of these glutamate receptors in benzodiazepine withdrawal signs, we summarize recent findings concerning i) the effects of ligands for each type of glutamate receptor on the expression of withdrawal signs caused by the discontinuation of chronic benzodiazepine treatment using behavioral approaches, and ii) the change in glutamate receptors during benzodiazepine withdrawal using biochemical approaches.

Role of NMDA receptors

The first experimental evidence on the role of NMDA receptor in benzodiazepine withdrawal signs was reported by Steppuhn and Turski (4), who showed that diazepam withdrawal responses (epileptiform activity, muscle tone and anxious behavior) can be suppressed by the intracerebral infusion of an antagonist for the glutamate binding site on the NMDA receptor (3-{[(±)-2-carboxy-piperazin-4-yl]-propyl-1-phosphonate (CPP)) when withdrawal signs appear. NMDA receptors have been shown to be ligand-gated ion channel complexes that have multiple regulatory sites (such as an ion-channel blocker binding site and a strychnine-insensitive glycine binding site) in addition to a glutamate binding site (5). We further examined the effect of antagonists for these other sites on NMDA receptors and found that blockers for the ion-channel site (dizocilpine) and the glycine binding site (7-chlorokynurenic acid) can also suppress the enhanced susceptibility to seizure during diazepam withdrawal (6, 7), which is an experimental model of benzodiazepine withdrawal signs in mice (8). These findings indicate that NMDA receptors play an important role in the expression of benzodiazepine withdrawal signs. Furthermore, in the above study (6, 7), we observed an interesting finding regarding the effect of ifenprodil, which is an antagonist for NMDAR2B (NR2B)-subunit-containing NMDA receptors. Ifenprodil alone did not protect against seizure caused by pentylentetrazole and methyl-6,7-dimethoxy-4-ethyl-β-carbolene-3-carboxylate (DMCM), which reduce the function of GABA receptors, but completely reversed the enhanced susceptibilities to pentylentetrazole- and DMCM-induced seizures in diazepam-withdrawn mice (7–9). Ifenprodil has been reported to bind not only to NMDA receptors but also to σ receptors (10); however, pretreatment with a σ-receptor agonist or antagonist fails to influence the hypersusceptibility to seizure in diazepam-withdrawn mice (7), suggesting that the blocking action of ifenprodil on NMDA receptors is responsible for the prevention of withdrawal-induced hypersusceptibility to seizure by ifenprodil. We have also found that ifenprodil also protects against seizure in neonatal mice (7- and 10-day-old) despite the absence of a similar effect in young adult mice (>21-day-old) (11). Several lines of evidence have shown that mRNA for the NR2B subunit is expressed at high levels in neonatal rodent brain, while that for the NR2A subunit, which is a low-affinity site for ifenprodil, is found at a low level in neonates and increases with age (12–14). In fact, we have demonstrated that the binding site for [3H]-dizocilpine, a radioligand for NMDA receptors, in a membrane preparation from neonatal mouse brain is almost completely displaced by a low concentration of ifenprodil (11), suggesting that there is a high population of high-affinity ifenprodil-binding sites on NMDA receptors (perhaps NR2B-containing receptors) in neonatal mice, in which ifenprodil has a potent antiseizure effect. Thus, the experimental evidence that ifenprodil effectively prevents withdrawal-induced hypersusceptibility to seizure leads to the hypothesis that the high-affinity ifenprodil-binding site (NR2B subunit)-containing NMDA receptors may be upregulated during diazepam withdrawal and that this change in NMDA receptors may be responsible for the hypersusceptibility to seizure caused by diazepam withdrawal. The putative upregulation of NMDA receptors during withdrawal has been supported by findings that the susceptibility to seizure caused by the intracerebroventricular administration of NMDA is enhanced in diazepam-withdrawn mice (4, 7).

The reliable demonstration and quantitative characterization of benzodiazepine withdrawal signs have been made feasible only by examining abrupt withdrawal from benzodiazepines. We have established a model of physical dependence on diazepam using the long-term consumption of diazepam-admixed food in Fischer 344 rats (15, 16). Only this model has been successfully used to induce severe benzodiazepine withdrawal signs that are also seen clinically such as anxiety, muscle spasms and seizures. Using this model, we have demonstrated that the NMDA receptor antagonist dizocilpine and ifenprodil can markedly suppress the spontaneous expression of diazepam withdrawal signs (17). This has strongly supported the proposal based on experiments using mice that NMDA receptor-mediated mechanisms are involved in the expression of diazepam withdrawal signs. It has been considered that benzodiazepine withdrawal signs are a heterogeneous phenomenon and involve several different underlying signs mediated by different mechanisms (18). We have observed 18 withdrawal signs using this model and have divided these signs (excluding “death”) into 4 groups (motor, emotional, and autonomic withdrawal signs and body weight loss) and re-analyzed the effects of NMDA-
receptor antagonists. The results of this re-analysis have shown that treatment with dizocilpine markedly suppresses motor withdrawal signs and body weight loss, while ifenprodil effectively suppresses the expression of motor and emotional withdrawal signs (17). Thus, only motor withdrawal signs are suppressed by both NMDA-receptor antagonists. Therefore, the signal for the excitatory amino acid system via NMDA receptors may mainly contribute to the expression of motor behavioral signs of diazepam withdrawal. This contention is consistent with a previous view (3, 19) that the role glutamatergic neurotransmission plays in benzodiazepine withdrawal is specific for convulsion and related withdrawal signs. However, we found that other behavioral signs could only be prevented by treatment with dizocilpine or ifenprodil (17). At present, although it remains unclear what receptors contribute to the prevention of withdrawal signs by dizocilpine or ifenprodil, the effects of these antagonists on different subunits of the NMDA receptor or other neurotransmitter receptors seem to be involved. As for body weight loss, which is dramatically suppressed by dizocilpine but not ifenprodil, it is conceivable that dizocilpine-sensitive and ifenprodil-insensitive NMDA receptors (perhaps NR2A-containing receptor) may be involved because dizocilpine has a high affinity for NR2A- and/or NR2B-subunit-containing NMDA receptors, while ifenprodil has very low affinity for the NR2A-subunit-containing NMDA receptor (5, 20). In emotional withdrawal signs, which are reduced by ifenprodil, it seems likely that such suppression by ifenprodil may be associated with not only the NMDA receptor but also with other neurotransmitter receptors such as adrenergic α-receptors, σ receptors and 5HT1 receptors (17). Using the above model of physical dependence resulting from long-term treatment with diazepam in rats, we have revealed two important pieces of evidence. First, the expression of each withdrawal sign is mediated by different receptor subtypes or neurotransmitter receptors. Second, the excitatory signal mediated by NMDA receptors plays a pivotal role in the expression of motor withdrawal signs.

The findings obtained in the above behavioral studies lead to the possibility that the excitatory signal via NMDA receptors may be enhanced during diazepam withdrawal. To clarify this possibility, we investigated the level of NMDA receptors by a receptor binding assay using [3H]-dizocilpine. Based on a Scatchard analysis of the results of a saturation binding experiment, the Bmax value of [3H]-dizocilpine binding sites, but not the Kd value, was greater in the membrane preparation from diazepam-withdrawn animals than in that from a control (7, 21). Interestingly, an increase in the Bmax value of [3H]-dizocilpine binding has been observed only in the membrane preparation from cerebral cortex, but not the hippocampus or cerebellum (21). These results indicate that the NMDA receptor is upregulated selectively in the cerebrocortical area when diazepam withdrawal signs appear after the discontinuation of diazepam treatment. We further demonstrated using a Western blot assay that the protein levels of the NR1 and NR2B subunits of NMDA receptors are increased in the membrane preparation from diazepam-withdrawn rat cerebral cortex (22). These findings suggest that the increase in the NR1 and NR2B subunits may be responsible for the upregulation of [3H]-dizocilpine binding in the cerebral cortex. The findings obtained from all of the above studies lead to the suggestion that the upregulation of both the NR1 and NR2B subunit proteins of the NMDA receptor in diazepam-withdrawn rat cerebral cortex is a neuroadaptive response resulting from chronic diazepam treatment and that the upregulation of NMDA receptors may play an important role in the expression of diazepam withdrawal signs (particularly motor withdrawal signs) (Fig. 1).

**Role of non-NMDA receptors**

Steppuhn and Turski (4) found that the susceptibilities to seizures caused by non-NMDA receptors agonists α-amino-3-hydroxy-5-β-ert-butyl-4-isoxazolepropionate (ATPA) and kainate were enhanced only at 2 and 3 days after the discontinuation of chronic diazepam treatment. Interestingly, in the model used here, diazepam withdrawal signs were not observed for the first 3 days after discontinuation (silent phase). Withdrawal signs appeared rapidly, peaked at withdrawal day 4, and recovered slowly up to day 21 (active phase). Therefore, the hypersensitivity of non-NMDA receptors has been shown to occur when obvious withdrawal signs do not appear. What is the physiological significance of the hypersensitivity of non-NMDA receptors during the silent phase in the expression of diazepam withdrawal signs? They have proposed that such hypersensitivity is important in triggering the expression of diazepam withdrawal signs during the active phase based on the following demonstration. Diazepam withdrawal responses (epileptiform activity, muscle tone and anxious behavior) are reduced by the intracerebral infusion of the AMPA-receptor antagonist 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 52466) during the silent phase; however, treatment with GYKI 52466 when withdrawal signs appear fails to produce similar suppression. Therefore, these findings suggest that the activation of non-NMDA receptors in the brain is essential for inducing withdrawal signs by the discontinuation of chronic diazepam treatment.
Role of metabotropic glutamate receptors (mGluR)

mGluR is currently divided into at least eight different subtypes (mGluR1–8) and classified into three subgroups (groups 1–3 mGluR) (23). We have found that the group 1 mGluR (mGluR1,2,3) antagonist (S)-4-carboxyphenylglycine ((S)-4CPG) has no antiseizure effect in vehicle-treated mice, but effectively blocks the hypersusceptibility to seizure in diazepam-withdrawn mice (24). A similar result has been observed in mice treated with the non-selective mGluR antagonist L-amino-3-phosphonopropionate (L-AP3) (25). The enhanced antiseizure effect of the group 1 mGluR antagonist in benzodiazepine-withdrawn mice has suggested the possibility that group 1 mGluR sensitivity may change. Group 1 mGluR has been reported to be associated with the phosphoinositide (PI)/calcium cascade (23). Mortensen et al. (25) supported this possibility by demonstrating that the stimulation of PI hydrolysis by the group 1 mGluR agonist (1S,3R)-1-aminocyclopentane dicarboxylic acid [(1S,3R)-ACPD] is increased in cortical slices from mice 2 and 3 days after discontinuing a 7-day exposure to lorazepam. These findings have suggested that the sensitivity of PI-coupled mGluR (group 1 mGluR) is enhanced, and excess activation of group 1 mGluR may be responsible for the hypersusceptibility to seizure in benzodiazepine-withdrawn mice (Fig. 1). This contention has been supported by findings that the activation of group 1 mGluR by a receptor agonist can potentiate seizure activity of pentylentetrazole (26) and that the upregulation of group 1 mGluR plays a role in epileptic disorders (27).

In contrast to the putative upregulation of group 1 mGluR, we have also found that the sensitivity of group 2 (mGluR2,3) and group 3 (mGluR4,6,7,8) mGluRs is reduced during diazepam withdrawal based on the following observation: agonists for group 2 mGluR, (2S,1,5,2)-2-(carboxycyclopropyl)-glycine (L-CCG-1) and L-+(+)-2-aminotetrahydrofuranobutyric acid (L-AP4), fail to suppress the hypersusceptibility to seizure in diazepam-withdrawn mice, even though these receptor agonists increase the seizure threshold in control mice (24). Group 2 and group 3 (mGluR4,6,7,8) mGluRs have been reported to negatively couple to adenyl cyclase, and recent studies have shown that group 2 and 3 mGluRs are mainly localized in presynaptic elements (28) and inhibit glutamatergic transmission (29, 30). Therefore, it is hypothesized that subsensitivity to group 2 and 3 mGluRs may lead to an increase in glutamate release, which in turn generates hypersusceptibility to seizure in diazepam-withdrawn mice (Fig. 1).

Fig. 1. Schema of the role of glutamate receptors in expression of benzodiazepine withdrawal signs.
Possibility of using ligands for glutamate receptors as therapeutic targets

The above behavioral and biochemical findings in animals lead to the possibility that NMDA, non-NMDA and mGlu receptor antagonists may be useful for treating signs of benzodiazepine withdrawal. However, the NMDA receptor antagonist dizocilpine has been reported to possess potent psychotomimetic properties (31). In contrast, ifenprodil has been shown not to produce the side effects that are associated with traditional NMDA receptor antagonists such as dizocilpine and phencyclidine (32). Our laboratory has demonstrated that rats do not exhibit physical dependence on ifenprodil (33). Based on the finding that the NR2B subunit of NMDA receptors, which interacts with ifenprodil, is upregulated in diazepam-withdrawn rats (22), ifenprodil may have important therapeutic potential as a palliative agent for treating benzodiazepine withdrawal signs. On the other hand, an antagonist for non-NMDA receptors may be useful for preventing the expression of benzodiazepine withdrawal signs, based on the findings of Steppuhn and Turski (4). Furthermore, we have detected no abnormal behaviors in mice treated intracerebroventricularly with the group 1 mGluR antagonist (S)-4CPG, even in mice treated with a relatively high dose (24). Thus, it is possible that the group 1 mGluR may be a target site for the treatment of benzodiazepine withdrawal signs.

Conclusion

In this article, we have summarized recent findings on the role of glutamate receptors in the expression of benzodiazepine withdrawal signs. Antagonists for NMDA, non-NMDA and group 1 mGluRs have shown the ability to potently suppress the withdrawal signs caused by the discontinuation of chronic benzodiazepine administration in mice and rats. During withdrawal, signal transduction mediated by NMDA, non-NMDA and group 1 mGluRs is upregulated, while that mediated by group 2 and 3 mGluRs is downregulated. These changes in the glutamate receptor system in the brain are some of the strongest potential mechanisms for the expression of benzodiazepine withdrawal signs (Fig. 1). Steppuhn and Turski (4) found that the time-courses of the hypersensitivity of NMDA and non-NMDA receptors are different. It would be interesting to investigate the time-course of the hypersensitivity of mGluR and ion-channel type (NMDA and non-NMDA) glutamate receptors, since group 1 mGluRs has been shown to regulate the function of ion-channel glutamate receptors (NMDA type) through the activation of an intracellular messenger such as protein kinase C (34) (Fig. 1). Moreover, further studies, like that of Tsuda et al. (17), will be necessary to analyze symptomatically the effects of drugs on withdrawal signs to elucidate the specific target sites that contribute to the expression of certain withdrawal signs.

REFERENCES

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