ELSEVIER

European Journal of Pharmacology 369 (1999) 163–168

ejp

Role of metabotropic glutamate receptors in the hypersusceptibility to pentylenetetrazole-induced seizure during diazepam withdrawal

Tsutomu Suzuki *, Norifumi Shimizu, Makoto Tsuda, Miho Soma, Miwa Misawa

Department of Pharmacology, School of Pharmacy, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

Received 5 January 1999; revised 2 February 1999; accepted 5 February 1999

Abstract

To clarify the contribution of metabotropic glutamate (mGlu) receptors in brain to benzodiazepine withdrawal signs, we now examine the effects in mice of selective ligands for three subgroups of mGlu receptor on the hypersusceptibility to pentylenetetrazole-induced seizure during diazepam withdrawal. The seizure threshold for pentylenetetrazole was significantly decreased by the discontinuation of chronic diazepam treatment. The decrease in the seizure threshold for pentylenetetrazole during diazepam withdrawal was significantly suppressed by intracerebroventricular (i.c.v.) pretreatment with the group 1 mGlu receptor antagonist, (*S*)-4-carboxyphenylglycine ((*S*)-4CPG: 56 and 100 nmol). These doses of (*S*)-4CPG did not alter the seizure threshold in chronically vehicle-treated (control) mice. Pretreatment i.c.v. with a presynaptic mGlu receptor agonist (the group 2 mGlu receptor agonist (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)-glycine (L-CCG-I: 3.0 and 5.6 nmol) and the group 3 mGlu receptor agonist, L-amino-4-phosphonobutyric acid (L-AP4: 3.0 and 5.6 nmol)) failed to suppress the decrease in seizure threshold in diazepam-withdrawn mice, but increased the seizure threshold in control mice. Pretreatment i.c.v. with the group 1 mGlu receptor antagonist/group 2 mGlu receptor agonist, (*S*)-4-carboxy-3-hydroxyphenyl-glycine ((*S*)-4C3HPG: 56 and 100 nmol), significantly increased the seizure threshold in control mice and suppressed the decrease in seizure threshold in diazepam-withdrawn mice. These findings suggest that enhancement of group 1 mGlu receptor function and a decline in both group 2 and group 3 mGlu receptor functions may play an important role in the hypersusceptibility to pentylenetetrazole-induced seizure during diazepam withdrawal.

Keywords: Diazepam withdrawal; Metabotropic glutamate receptor; (*S*)-4CPG ((*S*)-4-carboxyphenylglycine); L-CCG-I ((2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)-glycine); L-AP4 (L-amino-4-phosphonobutyric acid); (*S*)-4C3HPG ((*S*)-4-carboxy-3-hydroxyphenylglycine)

1. Introduction

Benzodiazepines are most commonly prescribed as psychoactive drugs for the treatment of anxiety and sleep disturbances. However, chronic administration of benzodiazepines is known to have several undesirable effects such as tolerance and physical dependence with withdrawal signs appearing when benzodiazepine treatment is interrupted abruptly. Characteristic withdrawal signs in humans are anxiety, delirium, muscle spasms, seizures, weight loss and even death (Levy, 1984; Woods et al., 1987, 1992; Lader, 1994). In experimental animals, withdrawal signs include spontaneous seizure, increased muscle tone and a decreased seizure threshold for convulsants (such as pentylenetetrazole) (Woods et al., 1987; Suzuki et al., 1992; Mizoguchi et al., 1994; Tsuda et al., 1997a,b, 1998a,e). It has been considered that these withdrawal signs may reflect hyperexcitability of the central nervous system (CNS) as a result of an adaptive response to chronic treatment with benzodiazepines.

Glutamate is a major excitatory neurotransmitter in the vertebrate CNS. Steppuhn and Turski (1993) first reported that the expression of diazepam withdrawal signs is potently suppressed by treatment with the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor antagonist, 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (GYKI52466), and the *N*-methyl-D-aspartate (NMDA) receptor antagonist, 3-[(±)-2-carboxypiperazin-4-yl]-propyl-1-phosphonate (CPP). Our previous study also showed that the NMDA receptor antagonists, dizocilpine (MK-801) and ifenprodil, suppress

* Corresponding author. Telefax: +81-3-5498-5787; E-mail: suzuki@hoshi.ac.jp

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00082-5

diazepam withdrawal signs in mice (Tsuda et al., 1997a, 1998c) and rats (Tsuda et al., 1998e). Furthermore, we recently demonstrated that up-regulation of NMDA receptor (Tsuda et al., 1998b,c) and NMDA receptor subunit (NR1 and NR2B) proteins in the cerebral cortex is produced in diazepam-withdrawn rats (Tsuda et al., 1998d). These findings suggest that NMDA and AMPA receptors may play an important role in the expression of withdrawal signs caused by the discontinuation of chronic diazepam treatment.

Glutamate acts not only on ionotropic glutamate receptors (such as the NMDA receptor), but also on metabotropic glutamate (mGlu) receptors. Metabotropic glutamate receptors are currently divided into at least eight different subtypes ($mGlu_1$ receptor–$mGlu_8$ receptor), and are classified into three subgroups (groups 1–3 mGlu receptor) based on similarities in amino acid sequence, transduction mechanisms and selectivity of agonists (Pin and Duvoisin, 1995). Group 1 mGlu receptors ($mGlu_1$, $mGlu_5$ receptor) are linked to the phosphoinositide/calcium cascade, while group 2 mGlu receptors ($mGlu_2$, $mGlu_3$ receptor) and group 3 mGlu receptors ($mGlu_4$, $mGlu_6$, $mGlu_7$, $mGlu_8$ receptor) are negatively linked to adenyl cyclase. In recent behavioral and electrophysiological studies, group 1 mGlu receptor antagonists and group 2 and 3 mGlu receptor agonists have been reported to suppress CNS hyperexcitability such as seizure (Sacaan and Schoppe, 1992; Tizzano et al., 1993, 1995; Dalby and Thomsen, 1996; Camon et al., 1998) and epilepsy (Burke and Hablitz, 1994; Attwell et al., 1995; Abdul-Ghani et al., 1996, 1997). However, there is no information available concerning the involvement of mGlu receptors in the expression of benzodiazepine withdrawal signs; i.e., CNS hyperexcitability resulting from the discontinuation of chronic benzodiazepine administration. Therefore, we examined the role of mGlu receptors in the hypersusceptibility to pentylenetetrazole-induced seizure during diazepam withdrawal, using the following mGlu receptor compounds: the group 1 mGlu receptor antagonist, (*S*)-4-carboxyphenylglycine ((*S*)-4CPG), the group 2 mGlu receptor agonist, (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)-glycine (L-CCG-I), the group 3 mGlu receptor agonist, L-amino-4-phosphonobutyric acid (L-AP4), and the group 1 mGlu receptor antagonist/group 2 mGlu receptor agonist, (*S*)-4-carboxy-3-hydroxyphenylglycine ((*S*)-4C3HPG).

2. Materials and methods

The present study was conducted in accordance with the Declaration of Helsinki and with the Guiding Principles for the Care and Use of Laboratory Animals, adopted by the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture of Japan.

2.1. Animals

Male ddY mice (20–22 g) were obtained from Tokyo Animal Laboratories (Tokyo, Japan). The animals were housed at a temperature of 22 ± 1°C with a 12 h light–dark cycle (light on 8:30 AM to 8:30 PM). Food and water were available ad libitum.

2.2. Chronic diazepam treatment

Mice were treated intraperitoneally (i.p.) with diazepam (16 mg kg^{-1}) or vehicle (9% Tween 80/saline) once a day for 7 days. The seizure threshold for pentylenetetrazole was evaluated 48 h after the last injection of diazepam or vehicle.

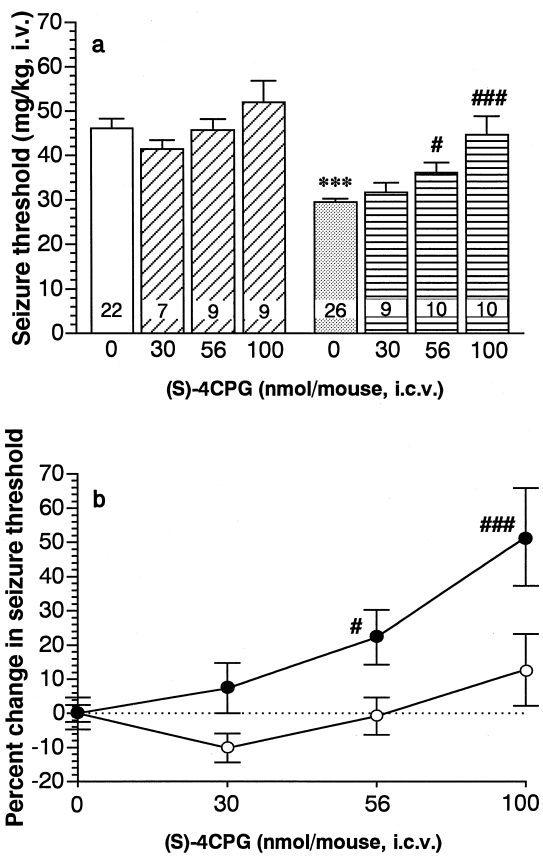

Fig. 1. (a) Effect of (*S*)-4CPG on the pentylenetetrazole-induced seizure threshold in chronically vehicle- or diazepam-treated mice. Ordinate: seizure threshold for pentylenetetrazole (mg kg^{-1}, i.v.). Chronic treatment: vehicle (open and diagonally-hatched columns) or diazepam (dotted and horizontally-hatched columns). (b) The percent change in pentylenetetrazole-induced seizure threshold in chronically vehicle- or diazepam-treated mice. Ordinate: percent change in pentylenetetrazole-induced seizure threshold. Chronic treatment: vehicle (open circle) or diazepam (closed circle). Each column and circle represents the mean with S.E.M. for 7–26 mice. Mice were injected with (*S*)-4CPG (30–100 nmol, i.c.v.) 15 min before pentylenetetrazole i.v. infusion. $^{***}P < 0.001$ vs. pretreatment with saline in the chronically vehicle-treated group. $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs. pretreatment with saline in the chronically diazepam-treated group.

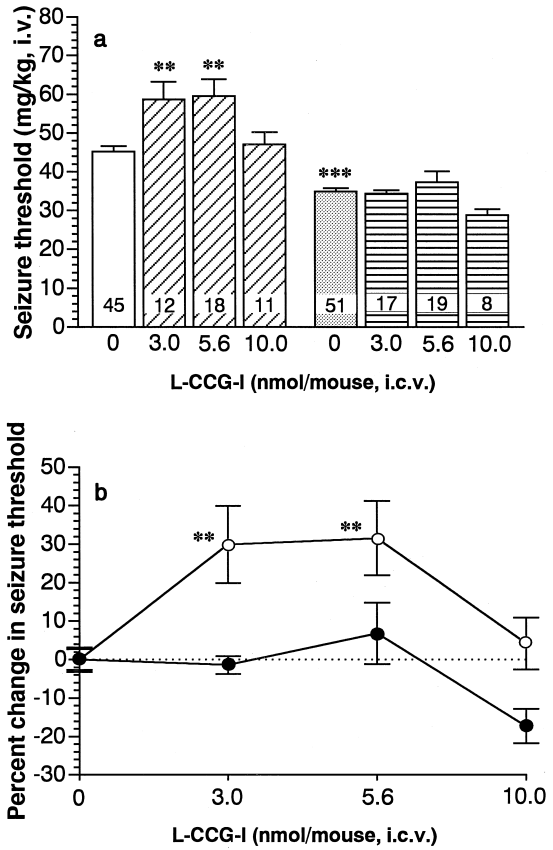

Fig. 2. (a) Effect of L-CCG-I on the pentylenetetrazole-induced seizure threshold in chronically vehicle- or diazepam-treated mice. Ordinate: seizure threshold for pentylenetetrazole (mg kg^{-1}, i.v.). Chronic treatment: vehicle (open and diagonally-hatched columns) or diazepam (dotted and horizontally-hatched columns). (b) The percent change in pentylenetetrazole-induced seizure threshold in chronically vehicle- or diazepam-treated mice. Ordinate: percent change in pentylenetetrazole-induced seizure threshold. Chronic treatment: vehicle (open circle) or diazepam (closed circle). Each column and circle represents the mean with S.E.M. for 8–51 mice. Mice were injected with L-CCG-I (3.0–10.0 nmol, i.c.v.) 15 min before pentylenetetrazole i.v. infusion. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. pretreatment with saline in the chronically vehicle-treated group.

2.3. Testing the seizure threshold for pentylenetetrazole

The threshold for pentylenetetrazole-induced seizure was determined as described previously (Tsuda et al., 1997a). Mice were placed in a Perspex cylinder (10 cm × 10 cm × 10 cm; $w \times l \times h$) and infused with pentylenetetrazole via the tail vein. The threshold for seizure was determined as the time to the first clonic convulsion lasting more than 1 s. Infusions were not given for more than 240 s. The rate of infusion was 0.23 ml min^{-1} for pentylenetetrazole, and the pentylenetetrazole concentration was adjusted to 5 mg ml^{-1}. Fifteen min before pentylenetetrazole infusion, the mice were injected intracerebroventricularly (i.c.v.) with (*S*)-4CPG (30–100 nmol), L-CCG-I (3.0–10.0 nmol), L-AP4 (1.0–5.6 nmol) or (*S*)-4C3HPG (56–100 nmol). The doses of these drugs were determined on the basis of their efficacy (Pin and Duvoisin, 1995). The i.c.v. injections were performed as described previously (Haley and McCormick, 1957; Suzuki et al., 1996). Briefly, one day before diazepam or vehicle treatment, the mice were anesthetized with ether and a 2-mm double-needle (tip: 27 gauge × 2 mm and base: 22 G × 10 mm, Natsume Seisakusyo, Tokyo, Japan) attached to a 25-μl Hamilton microsyringe was inserted into the unilateral injection site; as a result, a simple hole for the injection was made in the skull. When the seizure threshold for pentylenetetrazole was to be tested, the drugs were injected into the hole with the mice unanesthetized. The injection volume for i.c.v. injection was 5 μl. To avoid the fluctuations in the seizure threshold for pentylenetetrazole, the tests of different doses of each mGlu receptor ligand and vehicle (respective control) were performed between 1:00 and 3:00 PM within a week.

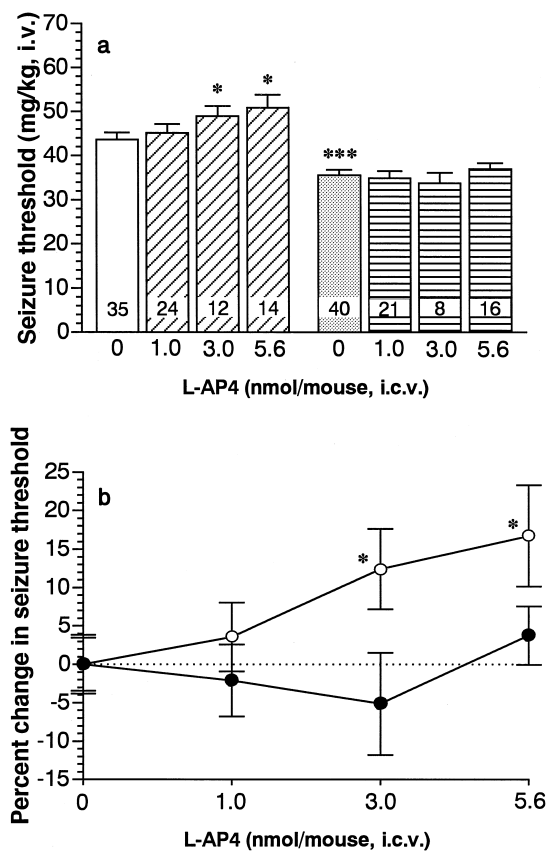

Fig. 3. (a) Effect of L-AP4 on the pentylenetetrazole-induced seizure threshold in chronically vehicle- or diazepam-treated mice. Ordinate: seizure threshold for pentylenetetrazole (mg kg^{-1}, i.v.). Chronic treatment: vehicle (open and diagonally-hatched columns) or diazepam (dotted and horizontally-hatched columns). (b) The percent change in pentylenetetrazole-induced seizure threshold in chronically vehicle- or diazepam-treated mice. Ordinate: percent change in pentylenetetrazole-induced seizure threshold. Chronic treatment: vehicle (open circle) or diazepam (closed circle). Each column and circle represents the mean with S.E.M. for 8–40 mice. Mice were injected with L-AP4 (1.0–5.6 nmol, i.c.v.) 15 min before pentylenetetrazole i.v. infusion. $^{*}P < 0.05$, $^{***}P < 0.001$ vs. pretreatment with saline in the chronically vehicle-treated group.

2.4. Drugs

Diazepam (Profarma, Italy) was suspended in vehicle consisting of 9% Tween-80 (Kishida Chemical, Osaka, Japan) in saline. Pentylenetetrazole (Sigma, St. Louis, USA), (*S*)-4CPG, L-CCG-I, L-AP4 and (*S*)-4C3HPG (Tocris Cookson, Bristol, UK) were dissolved in saline and adjusted with 1 N NaOH to a pH of approximately 7 to 8.

2.5. Statistical analysis

The seizure threshold data were evaluated with a Kruskal–Wallis analysis followed by Mann–Whitney *U*-tests.

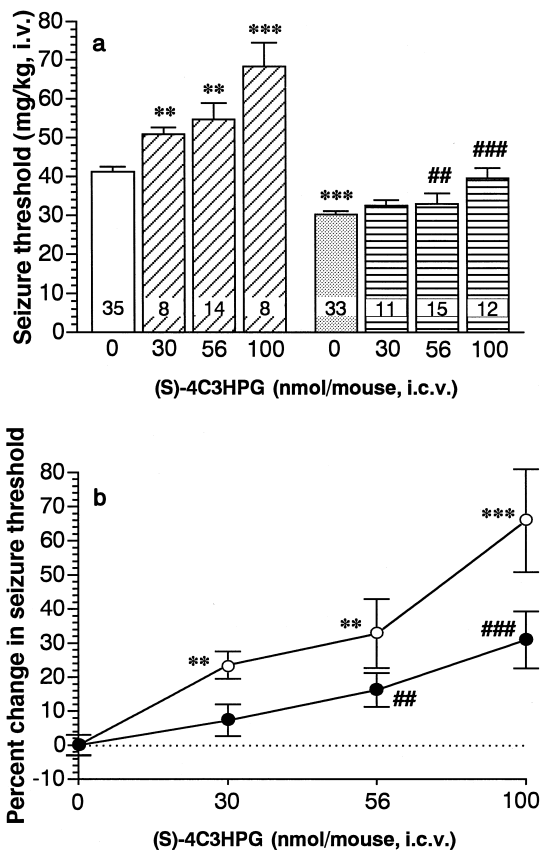

Fig. 4. (a) Effect of (*S*)-4C3HPG on the pentylenetetrazole-induced seizure threshold in chronically vehicle- or diazepam-treated mice. Ordinate: seizure threshold for pentylenetetrazole (mg kg^{-1}, i.v.). Chronic treatment: vehicle (open and diagonally-hatched columns) or diazepam (dotted and horizontally-hatched columns). (b) The percent change in pentylenetetrazole-induced seizure threshold in chronically vehicle- or diazepam-treated mice. Ordinate: percent change in pentylenetetrazole-induced seizure threshold. Chronic treatment: vehicle (open circle) or diazepam (closed circle). Each column and circle represents the mean with S.E.M. for 8–35 mice. Mice were injected with (*S*)-4C3HPG (30–100 nmol, i.c.v.) 15 min before pentylenetetrazole i.v. infusion. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. pretreatment with saline in the chronically vehicle-treated group. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. pretreatment with saline in the chronically diazepam-treated group.

3. Results

Withdrawal from chronic treatment with diazepam elicited a significant increase in the susceptibility to seizure with a decrease of approximately 20–35% ($P < 0.001$; Figs. 1–4) in seizure threshold. The decrease in seizure threshold for pentylenetetrazole during diazepam withdrawal was significantly suppressed by i.c.v. pretreatment with 56 ($P < 0.05$) or 100 nmol ($P < 0.001$) of (*S*)-4CPG (Fig. 1a), and the percent increases in seizure threshold were 22 and 52%, respectively (Fig. 1b). However, these doses of (*S*)-4CPG did not alter the seizure threshold for pentylenetetrazole in chronically vehicle-treated (control) mice. The seizure threshold for pentylenetetrazole in control mice was increased by i.c.v. pretreatment with 3.0 ($P < 0.01$) and 5.6 nmol ($P < 0.01$) of L-CCG-I (Fig. 2a), and the percent increases in seizure threshold were 30 and 32%, respectively (Fig. 2b). However, these doses of L-CCG-I did not alter the seizure threshold for pentylenetetrazole in diazepam-withdrawn mice. Treatment i.c.v. with 10 nmol of L-CCG-I did not influence the seizure threshold for pentylenetetrazole in control mice. The seizure threshold for pentylenetetrazole in control mice was increased by i.c.v. pretreatment with 3.0 ($P < 0.05$) and 5.6 nmol ($P < 0.05$) of L-AP4 (Fig. 3a), and the percent increases in seizure threshold were 12 and 17%, respectively (Fig. 3b). However, these doses of L-AP4 had no effect against the decrease in the seizure threshold for pentylenetetrazole in diazepam-withdrawn mice. The seizure threshold for pentylenetetrazole in control mice was increased by i.c.v. pretreatment with 10 (n.s.), 30 ($P < 0.01$), 56 ($P < 0.01$) and 100 nmol ($P < 0.001$) of (*S*)-4C3HPG (Fig. 4a) and the percent increases in seizure threshold were 8, 24, 33 and 66%, respectively (Fig. 4b). The decrease in the seizure threshold in diazepam-withdrawn mice was significantly suppressed by i.c.v. pretreatment with 56 ($P < 0.01$) and 100 nmol ($P < 0.001$) of (*S*)-4C3HPG (Fig. 4a) and the percent increases in the seizure threshold were 16 and 31%, respectively (Fig. 4b).

4. Discussion

The seizure threshold for pentylenetetrazole was significantly decreased when chronic diazepam treatment of mice was discontinued. This result is consistent with the findings from previous studies (Woods et al., 1987), and reflects withdrawal hyperexcitability in response to physical dependence.

The present results provided the first demonstration that mGlu receptors are involved in the expression of the benzodiazepine withdrawal response in vivo. We first demonstrated that the group 1 mGlu receptor antagonist, (*S*)-4CPG, dose-dependently suppresses the increase in seizure susceptibility to pentylenetetrazole in diazepam-withdrawn mice. This suppression by (*S*)-4CPG was ob-

served at doses which did not affect the seizure threshold in the control group. This finding suggests that the function of the group 1 mGlu receptor may change during diazepam withdrawal. In fact, Mortensen et al. (1995) recently demonstrated that phosphoinositide hydrolysis induced by the non-selective mGlu receptor agonist, 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD) is enhanced 2 days after discontinuation of chronic treatment with lorazepam in mice. Enhancement of the group 1 mGlu receptor response has also been observed in amygdala neurons from kindled animals which are a model for pathological seizure disorders (Holmes et al., 1996). As the activation of group 1 mGlu receptor produces seizure (Camon et al., 1998), excess activation of group 1 mGlu receptors may play an important role in the hypersusceptibility to pentylenetetrazole-induced seizure in diazepam-withdrawn mice.

Furthermore, it has been reported that group 1 mGlu receptors enhance NMDA receptor function as a result of the activation of protein kinase C (Pisani et al., 1997). We found that the decrease in the seizure threshold for pentylenetetrazole during diazepam withdrawal is suppressed by the NMDA receptor antagonists, dizocilpine and ifenprodil (Tsuda et al., 1997a). Therefore, the hypersusceptibility to pentylenetetrazole-induced seizure during diazepam withdrawal may also be responsible for the enhancement of NMDA receptor function as a result of the activation of group 1 mGlu receptors.

In contrast to the results with the group 1 mGlu receptor antagonist, (*S*)-4CPG, both the group 2 mGlu receptor agonist, L-CCG-I (3.0 and 5.6 nmol), and the group 3 mGlu receptor agonist, L-AP4, failed to suppress the hypersusceptibility to pentylenetetrazole-induced seizure in diazepam-withdrawn mice, even though these receptor agonists significantly increased the threshold for pentylenetetrazole-induced seizure in control mice. These results indicate that subsensitivity to group 2 and group 3 mGlu receptors may occur during diazepam withdrawal. Recent immunohistochemical and neurochemical studies have shown that group 2 and 3 mGlu receptors are mainly localized in presynaptic elements (Shigemoto et al., 1997) and that glutamatergic transmission is attenuated by the activation of group 2 and 3 mGlu receptors (Attwell et al., 1995; East et al., 1995). Therefore, subsensitivity to group 2 and 3 mGlu receptors may lead to an increase in glutamate release, which in turn triggers hypersusceptibility to pentylenetetrazole-induced seizure during diazepam withdrawal. This hypothesis is supported by previous findings that glutamate release is increased during withdrawal from other sedative drugs such as ethanol (Rossetti and Carboni, 1995) and phenobarbital (Tanaka et al., 1996). However, this hypothesis may need further investigation.

Surprisingly, when mice were pretreated with a high dose (10 nmol) of L-CCG-I, the increase in the seizure threshold for pentylenetetrazole in control mice disappeared. Although the exact reasons for this result remain to be elucidated, it is hypothesized that the antiseizure effect of a high dose of L-CCG-I may be masked by its agonistic action on group 1 mGlu receptors (Pin and Duvoisin, 1995) or a reduction in γ-aminobutyric acid (GABA) release via activation of group 2 mGlu receptors (Hayashi et al., 1993; Poncer et al., 1995).

These findings suggest that group 1 mGlu receptor antagonists especially inhibit the decrease in the seizure threshold for pentylenetetrazole in diazepam-withdrawn mice while group 2 mGlu receptor agonists especially increase the seizure threshold in chronically vehicle-treated mice. This assumption is further supported by the finding that (*S*)-4C3HPG, which is a group 1 mGlu receptor antagonist/group 2 mGlu receptor agonist (Hayashi et al., 1994; Pin and Duvoisin, 1995), increased the seizure threshold in both chronically vehicle-treated mice and diazepam-withdrawn mice. Thus, the inhibition of the decrease in the seizure threshold in diazepam withdrawn-mice may be due to its antagonistic action on group 1 mGlu receptors and the increase in the seizure threshold in chronically vehicle-treated mice may be due to its agonistic action on group 2 mGlu receptors.

The signs of benzodiazepine withdrawal appear to be prevented by NMDA receptor antagonists such as dizocilpine and CPP (Steppuhn and Turski, 1993; Tsuda et al., 1997a, 1998c,e), but these compounds produce a variety of adverse neurobehavioral effects (Koek et al., 1988; Tricklebank et al., 1989; Koek and Colpaert, 1990; Willetts et al., 1990). In the present experiment, we detected no such abnormal behavior on observation, even of mice treated with the highest dose of (*S*)-4CPG. Therefore, group 1 mGlu receptor antagonists may improve benzodiazepine withdrawal signs with no serious side-effects.

In conclusion, we have provided pharmacological evidence that the enhancement of group 1 mGlu receptor function and a decline in both group 2 and group 3 mGlu receptor function may play an important role in the hypersusceptibility to pentylenetetrazole-induced seizure during diazepam withdrawal. Thus, group 1 mGlu receptor antagonists may have a therapeutic potential as palliative agents for benzodiazepine withdrawal signs.

Acknowledgements

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Sciences, Sports and Culture and a Research Grant from the Ministry of Health and Welfare of Japan to T.S. We wish to thank Ms. Aya Myoga for her expert technical assistance.

References

Abdul-Ghani, A.-S., Attwell, P.J.E., Bradford, H.F., 1996. The antiepileptic effect of 3-aminopropylarsonate on electrically kindled amygdala. Brain Res. 742, 305–312.

Abdul-Ghani, A.-S., Attwell, P.J.E., Kent, N.S., Bradford, H.F., Croucher, M.J., Jane, D.E., 1997. Anti-epileptogenic and anticonvulsant activity of L-2-amino-4-phosphonobutyrate, a presynaptic glutamate receptor agonist. Brain Res. 755, 202–212.

Attwell, P.J.E., Kaura, S., Sigala, G., Bradford, H.F., Croucher, M.J., Jane, D.E., Watkins, J.C., 1995. Blockade of both epileptogenesis and glutamate release by (1*S*,3*S*)-ACPD, a presynaptic glutamate receptor agonist. Brain Res. 698, 155–162.

Burke, J.P., Hablitz, J.J., 1994. Metabotropic glutamate receptor activation decreases epileptiform activity in rat neocortex. Neurosci. Lett. 174, 29–33.

Camon, L., Vives, P., Devera, N., Martinez, E., 1998. Seizure and neuronal damage induced in the rat by activation of group I metabotropic glutamate receptors with their selective agonist 3,5-dihydroxyphenylglycine. J. Neurosci. Res. 51, 339–348.

Dalby, N.O., Thomsen, C., 1996. Modulation of seizure activity in mice by metabotropic glutamate receptor ligands. J. Pharmacol. Exp. Ther. 276, 516–522.

East, S.J., Hill, M.P., Brotchie, J.M., 1995. Metabotropic glutamate receptor agonists inhibit endogenous glutamate release from rat striatal synaptosomes. Eur. J. Pharmacol. 277, 117–121.

Haley, T.J., McCormick, W.G., 1957. Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. Br. J. Pharmacol. 12, 12–15.

Hayashi, Y., Momiyama, A., Takahashi, T., Ohishi, H., Ogawa-Megro, R., Shigemoto, R., Mizuno, N., Nakanishi, S., 1993. Role of a metabotropic glutamate receptor in synaptic modulation in the accessory olfactory bulb. Nature 366, 687–690.

Hayashi, Y., Sekeyama, N., Nakanishi, S., Jane, D.E., Sunter, D.C., Birse, E.F., Udvarhelyi, P.M., Watkins, J.C., 1994. Analysis of agonist and antagonist activities of phenylglycine derivatives for different cloned metabotropic glutamate receptor subtypes. J. Neurosci. 14, 3370–3377.

Holmes, K.H., Keele, N.B., Shinnick-Gallagher, P., 1996. Loss of mGluR mediated hyperpolarizations and increase in mGluR depolarizations in basolateral amygdala neurons in kindling induced epilepsy. J. Neurophysiol. 76, 2808–2812.

Koek, W., Colpaert, F.C., 1990. Selective blockade of *N*-methyl-D-aspartate (NMDA)-induced convulsions by NMDA antagonists and putative glycine antagonists: relationship with phencyclidine-like behavioral effects. J. Pharmacol. Exp. Ther. 252, 349–357.

Koek, W., Woods, J.H., Winger, G.D., 1988. MK-801, a proposed noncompetitive antagonist of excitatory amino acid neurotransmission, produces phencyclidine-like behavioral effects in pigeons, rats and rhesus monkeys. J. Pharmacol. Exp. Ther. 245, 969–974.

Lader, M., 1994. Anxiolytic drugs: dependence, addiction and abuse. Eur. Neropsychopharmacol. 4, 85–89.

Levy, A.B., 1984. Delirium and seizures due to abrupt alprazolam withdrawal: case report. J. Clin. Psychiatry 45, 38–39.

Mizoguchi, H., Shirayama, N., Tsuda, M., Yoshiike, M., Suzuki, T., Misawa, M., 1994. Potentiation of physical dependence on diazepam by ondansetron in rats. Life. Sci. 54, PL131–PL136.

Mortensen, M., Suzdak, P.D., Thomsen, C., 1995. The effect of lorazepam tolerance and withdrawal on metabotropic glutamate receptor function. J. Pharmacol. Exp. Ther. 274, 155–163.

Pin, J.-P., Duvoisin, R., 1995. The metabotropic glutamate receptors: structure and functions. Neuropharmacology 34, 1–26.

Pisani, A., Calabresi, P., Centonze, D., Bernardi, G., 1997. Enhancement of NMDA responses by group I metabotropic glutamate receptor activation in striatal neurones. Br. J. Pharmacol. 120, 1007–1014.

Poncer, J.C., Shinozaki, H., Miles, R., 1995. Dual modulation of synaptic inhibition by distinct metabotropic glutamate receptors in the rat hippocampus. J. Physiol. 485, 121–134.

Rossetti, Z.L., Carboni, S., 1995. Ethanol withdrawal is associated with increased extracellular glutamate in the rat striatum. Eur. J. Pharmacol. 283, 177–183.

Sacaan, A.I., Schoppe, D.D., 1992. Activation of hippocampal metabotropic excitatory amino acid receptors leads to seizures and neuronal damage. Neurosci. Lett. 139, 77–82.

Shigemoto, R., Kinoshita, A., Wada, E., Nomura, S., Ohishi, H., Takada, M., Flor, P.G., Neki, A., Abe, T., Nakanishi, S., Mizuno, N., 1997. Differential presynaptic localization of metabotropic glutamate receptor subtypes in the rat hippocampus. J. Neurosci. 17, 7503–7522.

Steppuhn, K.G., Turski, L., 1993. Diazepam dependence prevented by glutamate antagonists. Proc. Natl. Acad. Sci. USA 90, 6889–6893.

Suzuki, T., Lu, M.-S., Motegi, H., Yoshii, T., Misawa, M., 1992. Genetic differences in the development of physical dependence upon diazepam in Lewis and Fischer 344 inbred rat strains. Pharmacol. Biochem. Behav. 43, 387–393.

Suzuki, T., Tsuji, M., Mori, T., Misawa, M., Nagase, H., 1996. The effects of dopamine D_1 and D_2 receptor antagonists on the rewarding effects of δ_1 and δ_2 opioid receptor agonists in mice. Psychopharmacology 124, 211–218.

Tanaka, S., Kiuchi, Y., Tsuchida, A., Yamamoto, T., Oguchi, K., Yoshida, T., Kuroiwa, Y., 1996. Possible involvement of increase in brain extracellular concentration of excitatory amino acids in convulsion of phenobarbital-withdrawn rats. Res. Commun. Alcohol Subst. Abuse 17, 91–102.

Tizzano, J.P., Griffey, K.I., Johnson, J.A., Fix, A.S., Helton, D.R., Schoepp, D.D., 1993. Intracerebral 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD) produces limbic seizures that are not blocked by ionotropic glutamate receptor antagonists. Neurosci. Lett. 162, 12–16.

Tizzano, J.P., Griffey, K.I., Schoepp, D.D., 1995. Induction or protection of limbic seizures in mice by mGluR subtype selective agonists. Neuropharmacology 34, 1063–1067.

Tricklebank, M.D., Singh, L., Oles, R.J., Preston, C., Iversen, S.D., 1989. The behavioural effects of MK-801: a comparison with antagonists acting non-competitively and competitively at the NMDA receptor. Eur. J. Pharmacol. 167, 127–135.

Tsuda, M., Suzuki, T., Misawa, M., 1997a. Recovery of decreased seizure threshold for pentylenetetrazole during diazepam withdrawal by NMDA receptor antagonists. Eur. J. Pharmacol. 324, 63–66.

Tsuda, M., Suzuki, T., Misawa, M., 1997b. Modulation of the decrease in the seizure threshold of pentylenetetrazole in diazepam-withdrawn mice by the neurosteroid 5α-pregnan-3α, 21-diol 20-one (allo THDOC). Addict. Biol. 2, 445–460.

Tsuda, M., Shimizu, N., Yajima, Y., Suzuki, T., Misawa, M., 1998a. Role of nitric oxide in the hypersusceptibility to pentylenetetrazole-induced seizure in diazepam-withdrawn mice. Eur. J. Pharmacol. 344, 27–30.

Tsuda, M., Suzuki, T., Misawa, M., 1998b. Region-specific changes in [^{3}H]dizocilpine binding in diazepam-withdrawn rats. Neurosci. Lett. 240, 113–115.

Tsuda, M., Shimizu, N., Yajima, Y., Suzuki, T., Misawa, M., 1998c. Hypersusceptibility to DMCM-induced seizures during diazepam withdrawal in mice: evidence for upregulation of NMDA receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 357, 309–315.

Tsuda, M., Chiba, Y., Suzuki, T., Misawa, M., 1998d. Upregulation of NMDA receptor subunit proteins in the cerebral cortex during diazepam withdrawal. Eur. J. Pharmacol. 341, R1–R2.

Tsuda, M., Suzuki, T., Misawa, M., 1998e. NMDA receptor antagonists potently suppress the spontaneous withdrawal signs induced by discontinuation of long-term diazepam treatment in Fischer 344 rats. Brain Res. 790, 82–90.

Willetts, J., Balster, R.L., Leander, J.D., 1990. The behavioral pharmacology of NMDA receptor antagonists. Trends Pharmacol. Sci. 11, 423–428.

Woods, J.H., Katz, J.L., Winger, G., 1987. Abuse liability of benzodiazepines. Pharmacol. Rev. 39, 251–413.

Woods, J.H., Katz, J.L., Winger, G., 1992. Benzodiazepines: use, abuse and consequences. Pharmacol. Rev. 44, 151–347.