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Short communication

Recovery of decreased seizure threshold for pentylenetetrazole during diazepam withdrawal by NMDA receptor antagonists

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Abstract

The effects of several NMDA receptor antagonists on pentylenetetrazole-induced diazepam-withdrawal seizure were examined in mice. The decrease in the seizure threshold for pentylenetetrazole during diazepam withdrawal was inhibited by pretreatment with MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenzo(*a*,*d*)cycloheptan-5,10-imine maleate), 7-chlorokynurenic acid and ifenprodil. Furthermore, MK-801 and ifenprodil, at doses which did not affect the threshold of pentylenetetrazole-induced seizure in control mice, also significantly suppressed the decrease in the seizure threshold during diazepam withdrawal, whereas 7-chlorokynurenic acid did not. These findings suggest that overactivity of an ion channel site and an ifenprodil binding site on the NMDA receptor may play an important role in the hypersensitivity of pentylenetetrazole-induced seizure in diazepam-withdrawn mice.

Keywords: NMDA receptor; MK-801; 7-Chlorokynurenic acid; Ifenprodil; Diazepam withdrawal; (Mouse)

1. Introduction

Benzodiazepines are most commonly prescribed as psychoactive drugs for the treatment of disorders such as anxiety and sleep disturbances. The most important disadvantage of benzodiazepines is the development of dependence in many patients. Clinical experiments have shown that anxiety, muscle spasms, and seizure are major signs of dependence seen after the discontinuation of treatment with benzodiazepines. In experimental animals, withdrawal signs include body weight loss, spontaneous seizure, increased muscle tone and decreased seizure threshold for convulsants (such as pentylenetetrazole) (Woods et al., 1987; Mizoguchi et al., 1994).

It is generally considered that the neurophysiological activity of the mammalian brain is maintained by the balance between excitatory (such as glutamate) and inhibitory (such as GABA (γ-aminobutyric acid)) neurotransmission. Previous studies in rodents have shown that $GABA_A$ receptor function is decreased by the chronic administration of benzodiazepines, which is thought to be responsible for the development of benzodiazepine tolerance (Miller et al., 1988a). In contrast, when withdrawal signs appear in benzodiazepine-dependent animals, $GABA_A$ receptor function is already out of the depressed and down-regulated state (Miller et al., 1988b; Allan et al., 1992). Furthermore, hypersensitivity to a benzodiazepine inverse agonist and pentylenetetrazole during withdrawal has also been reported (Woods et al., 1987; Mizoguchi et al., 1993). As for excitatory neurotransmission, Steppuhn and Turski (1993) have reported that withdrawal signs in diazepam-dependent mice were suppressed by pretreatment with a competitive NMDA receptor antagonist, and that the seizure threshold for *N*-methyl-D-aspartate (NMDA) decreased markedly after discontinuation of chronic diazepam treatment in mice. These findings suggest that, in addition to $GABA_A$ receptor function, enhancement of NMDA receptor function may be associated with the expression of withdrawal signs.

The NMDA receptor is a ligand-gated ion channel complex that has multiple regulatory sites in addition to the glutamate recognition site. These sites include an ion-channel blocker (such as MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenzo(*a*,*d*)cycloheptan-5,10-imine maleate)) binding site, a strychnine-insensitive glycine binding site and a polyamine binding site (Johnson and Ascher, 1987; Williams et al., 1991). However, there is little information available on the effects of selective ligands for these latter three sites of the NMDA receptor on benzodiazepine withdrawal seizure. Therefore, to clarify the role of the NMDA

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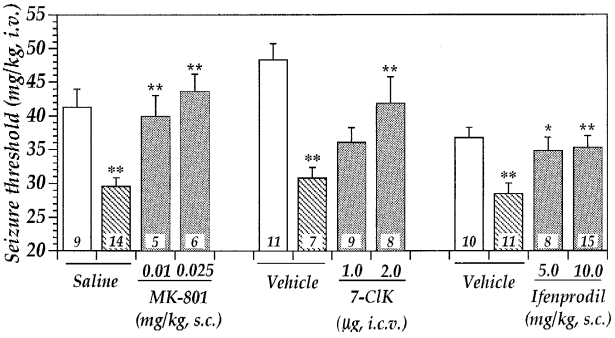

Fig. 1. Effect of each NMDA receptor antagonist on the decrease in the threshold for pentylenetetrazole-induced seizure during diazepam withdrawal in mice. Mice were injected with MK-801 (0.01 and 0.025 mg/kg, s.c.), 7-chlorokynurenic acid (1.0 and 2.0 μg/mouse, i.c.v.) and ifenprodil (5.0 and 10.0 mg/kg, i.p.) 30, 15 and 30 min before pentylenetetrazole i.v. infusion, respectively. Chronic treatment: vehicle (open columns) or diazepam (hatched and dotted columns). Ordinate: seizure threshold for pentylenetetrazole (mg/kg, i.v.). Each column represents the mean with S.E.M. for 8–15 mice. ** $P < 0.01$ vs. chronically vehicle-treated group. * $P < 0.05$, ** $P < 0.01$ vs. pretreatment with vehicle in chronically diazepam-treated group.

receptor in the expression of benzodiazepine withdrawal signs, we examined the effects of antagonists for distinct binding sites on the NMDA receptor on the decrease in the threshold for pentylenetetrazole-induced seizure during diazepam withdrawal in mice.

2. Materials and methods

2.1. Animals

Male *ddY* mice (20–23 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 a.m. to 8:30 p.m.). Food and water were available ad libitum.

2.2. Chronic diazepam treatment

Mice were treated i.p. with diazepam (16 mg/kg) or vehicle (9% Tween 80/saline) once a day for 6 days. The seizure threshold for pentylenetetrazole was evaluated 48 h after the last injection of diazepam.

2.3. Testing the seizure threshold for pentylenetetrazole

Mice were placed in a Perspex cylinder (10 × 10 × 10 cm; w × l × h) and infused with pentylenetetrazole via the tail vein. The threshold for seizure was determined as the time to the first clonic convulsion with a duration of more than 1 s. Infusions were not given for more than 240 s. The rate of infusion was 0.23 ml/min for pentylenetetrazole, and the pentylenetetrazole concentration was adjusted to 5 mg/ml. Mice were injected s.c. with MK-801 (0.01 and 0.025 mg/kg) 30 min before pentylenetetrazole infusion. 7-Chlorokynurenic acid (1.0 and 2.0 μg/mouse, i.c.v.) and ifenprodil (5 and 10 mg/kg, i.p.) were injected 15 and 30 min before pentylenetetrazole infusion, respectively. The injection volume for i.c.v. injection was 5 μl.

2.4. Drugs

Diazepam (Profarma, Italy) was suspended in vehicle consisting of 9% Tween 80 (Kishida, Osaka, Japan) in saline. Pentylenetetrazole (Sigma, St. Louis, MO, USA) and MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenzo(*a*,*d*)cycloheptan-5,10-imine maleate; Merck/Banyu, Tokyo, Japan) were dissolved in saline. 7-Chlorokynurenic acid (Research Biochemicals International, Natick, MA, USA) was dissolved in 1 M NaOH; the pH was adjusted to 6.5–7.5 with 0.05 M HCl before use. Ifenprodil (Research Biochemicals International) was dissolved in DMSO (dimethyl sulfoxide) and diluted in 5% DMSO with 9% Tween 80/saline before use.

2.5. Statistical analysis

The seizure threshold was evaluated statistically with Student's *t*-test or the non-parametric Wilcoxon test.

3. Results

The effect of diazepam withdrawal on seizure susceptibility was tested by tail vein infusion of pentylenetetrazole. Withdrawal from chronic treatment with diazepam elicited a significant increase in seizure susceptibility with a decrease of approximately 23–40% ($P < 0.01$; Fig. 1) in the seizure threshold. The decrease in the seizure threshold for pentylenetetrazole during diazepam withdrawal was significantly inhibited by pretreatment with MK-801 (0.01 and 0.025 mg/kg, s.c.) ($P < 0.01$; Fig. 1). In chronically

Table 1
Effect of each NMDA receptor antagonist on the pentylenetetrazole-induced seizure in chronic vehicle-treated mice

Drug	Dose	Seizure threshold (mg/kg, i.v.)	*n*
Saline		41.3 ± 2.7	9
MK-801 (mg/kg)	0.01	45.9 ± 2.6	7
	0.025	55.1 ± 3.3 [a]	10
Vehicle		41.4 ± 1.1	10
7-ClK (μg)	1.0	51.3 ± 4.9	9
	2.0	56.1 ± 3.2 [a]	15
Vehicle		36.6 ± 1.6	10
Ifenprodil (mg/kg)	10.0	39.7 ± 3.0	8

Mice were injected with MK-801 (s.c.), 7-chlorokynurenic acid (7-ClK) (i.c.v.) and ifenprodil (i.p.) 30, 15 and 30 min before pentylenetetrazole infusion, respectively. Each value represents the mean with S.E.M. for 7–15 mice.
[a] $P < 0.01$ vs. vehicle-treated group.

vehicle-treated mice, 0.025 mg/kg, but not 0.01 mg/kg, MK-801 increased the threshold for pentylenetetrazole-induced seizure ($P < 0.01$; Table 1). Furthermore, the glycine site antagonist, 7-chlorokynurenic acid (2 μg/mouse, i.c.v.), protected against the decrease in the seizure threshold for pentylenetetrazole in diazepam-withdrawn mice ($P < 0.01$; Fig. 1), and increased the seizure threshold for pentylenetetrazole in vehicle-treated mice ($P < 0.01$; Table 1). In contrast, pretreatment with the polyamine site non-competitive antagonist, ifenprodil (10 mg/kg, i.p.), which did not alter the seizure threshold for pentylenetetrazole in the control (Table 1), significantly reversed the decrease in the seizure threshold for pentylenetetrazole in diazepam-withdrawn mice ($P < 0.01$; Fig. 1).

4. Discussion

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

We first demonstrated that MK-801, an NMDA receptor antagonist for the ion-channel site, completely suppressed the increase in seizure susceptibility for pentylenetetrazole in diazepam-withdrawn mice. Furthermore, this suppression was seen even at a dose of 0.01 mg/kg of MK-801, which had no significant effect on the seizure threshold for pentylenetetrazole in chronically vehicle-treated mice. This effect of MK-801 to block pentylenetetrazole-induced seizure in diazepam-withdrawn mice was also seen in its inhibition of the NMDA-induced increase in firing rate of noradrenergic locus coeruleus neurons in ethanol-withdrawn rats (Engberg and Hajos, 1992). Furthermore, [^{3}H]MK-801 binding in the brain is increased by the discontinuation of chronic ethanol treatment in mice (Gulya et al., 1991). These findings led to the hypothesis that overactivity of NMDA receptor function may also occur during diazepam withdrawal. This hypothesis was supported by a previous finding that the seizure susceptibility to NMDA was enhanced during diazepam withdrawal in mice (Steppuhn and Turski, 1993). In this context, the overactivity of NMDA receptor function may trigger the increased susceptibility of pentylenetetrazole-induced seizure during diazepam withdrawal.

Numerous studies have suggested that there are at least two modulatory sites for endogenous ligands on the NMDA receptor: a strychnine-insensitive glycine binding site and a polyamine binding site. Therefore, we investigated whether the glycine site competitive antagonist, 7-chlorokynurenic acid, and the polyamine site non-competitive antagonist, ifenprodil, would block the increased susceptibility to pentylenetetrazole-induced seizure during diazepam withdrawal. The decrease in the seizure threshold for pentylenetetrazole was significantly blocked by pretreatment with 7-chlorokynurenic acid (2 μg/mouse, i.c.v.). However, since 7-chlorokynurenic acid (2 μg/mouse, i.c.v.) also increased the seizure threshold for pentylenetetrazole in the control group, while 7-chlorokynurenic acid (1 μg/mouse, i.c.v.) had no effect in either group, the blockade by 7-chlorokynurenic acid of the increased susceptibility to pentylenetetrazole-induced seizure in diazepam-withdrawn mice might have been due to functional antagonism by the anticonvulsant effect of 7-chlorokynurenic acid. In contrast, ifenprodil, which did not affect the seizure threshold for pentylenetetrazole in the chronically vehicle-treated group, suppressed the increased susceptibility to pentylenetetrazole-induced seizure. It is known that ifenprodil binds several receptors such as the NMDA receptor and σ site. We recently demonstrated that the sensitivity of the σ site agonist, (+)-pentazocine, and the σ site antagonist, NE-100 (*N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]-ethylamine monohydrochloride), did not change during diazepam withdrawal (Tsuda et al., in preparation). Therefore, the ifenprodil binding site on the NMDA receptor may also be upregulated by the discontinuation of chronic diazepam administration. In a recent study with a cortical culture of rat brain neurons, the inhibition of NMDA-stimulated Ca^{2+} flux by ifenprodil was enhanced by chronic ethanol exposure, suggesting that the expression of ifenprodil-sensitive NMDA receptor subunits (NR1/NR2B) may have been enhanced (Blevins et al., 1995). It is hypothesized that MK-801- and ifenprodil-sensitive NMDA receptors (perhaps NR1/NR2B-containing receptors) may be upregulated during diazepam withdrawal, and that this upregulation may lead to overactivity of NMDA receptor function, which in turn results in hypersensitivity to pentylenetetrazole-induced seizure.

In our laboratory, it has been previously demonstrated that the expression of withdrawal signs in phenobarbital-dependent rats was markedly suppressed by oral administration of ifenprodil (Yanaura et al., 1978). Furthermore, Gotti et al. (1988) reported that ifenprodil does not produce the motor and cognitive side effects associated with traditional NMDA receptor antagonists. Therefore, it is possible that ifenprodil may have important therapeutic potential as a palliative agent for benzodiazepine withdrawal signs.

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