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Upregulation of NMDA receptor subunit proteins in the cerebral cortex during diazepam withdrawal

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Abstract

The present study investigated the changes in NMDA receptor subunit proteins in diazepam-withdrawn rat cerebral cortex, using Western blotting analysis. The protein levels of the NR1 and NR2B, but not NR2A, subunits were significantly increased in diazepam-withdrawn rats compared to those in control rats. Therefore, an increase in the NR1 and NR2B subunit proteins may be responsible for both the previously observed upregulation of [^{3}H]dizocilpine binding in the cerebral cortex and the appearance of diazepam withdrawal signs. © 1998 Elsevier Science B.V.

Keywords: Diazepam withdrawal; NMDA receptor subunit; Cerebral cortex

The discontinuation of chronic benzodiazepine administration results in withdrawal signs characterized by anxiety, seizure and even mortality (Woods et al., 1992). We recently demonstrated (1) the potent suppression of diazepam withdrawal signs by NMDA receptor antagonists (Tsuda et al., 1997b) and (2) the selective upregulation of [^{3}H]dizocilpine binding sites in the cerebral cortex during diazepam withdrawal (Tsuda et al., submitted). These findings lead to the suggestion that the NMDA receptor may play an important role in the appearance of benzodiazepine withdrawal signs. Recently, two families of brain NMDA receptors, NMDAR1 (NR1) and NMDAR2 (NR2; A, B, C and D), have been cloned (Mori and Mishina, 1995). The expression of NR1 along with different NR2 subunits yields receptors with distinct pharmacological and physiological characteristics (Mori and Mishina, 1995). Several line of evidences have suggested that the ion-channel blocker dizocilpine shows high affinity for recombinant heteromeric NR1–NR2A and NR1–NR2B NMDA receptors (Laurie and Seeburg, 1994; Mori and Mishina, 1995). Therefore, to elucidate the changes in NMDA receptor subunits during diazepam withdrawal, in the present study we investigated the protein levels of the NR1, NR2A and NR2B subunits in the cerebral cortex of diazepam-withdrawn rats.

Male Fischer 344 rats (200–220 g) were used. Animals were housed in individual cages (24 × 21 × 15 cm: w × l × h) under a 12 h light–dark cycle (lights on from 8.30 to 20.30) with free access to food and tap water. Each rat was allowed to eat diazepam-admixed food (from 1 to 12 mg/g of food) or normal food for 30 days according to the method of Suzuki et al. (1992). Abrupt withdrawal was induced by substituting normal food for diazepam-admixed food on the last day of treatment. Diazepam-withdrawn rats were decapitated 42–45 h after the discontinuation of diazepam treatment. Cerebrocortical membranes from normal control and diazepam-withdrawn rats were prepared as previously described (Tsuda et al., 1997a). After protein determination (Lowry et al., 1951), the membrane fractions were solubilized and 20-μg aliquots were subjected to 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins were transferred electrophoretically to nitrocellulose membrane. After blocking, blots were incubated with anti-goat NR1, NR2A or NR2B polyclonal antibodies (1:500; Santa Cruz, USA). The blots were detected using a chemiluminescence method (ECL system; Amersham), and exposed to autoradiography films (Hyperfilm-ECL; Amersham). Quantitation of immunoreactive bands on the film was performed by image analysis (ATTO Densitograph Software). The values for diazepam-

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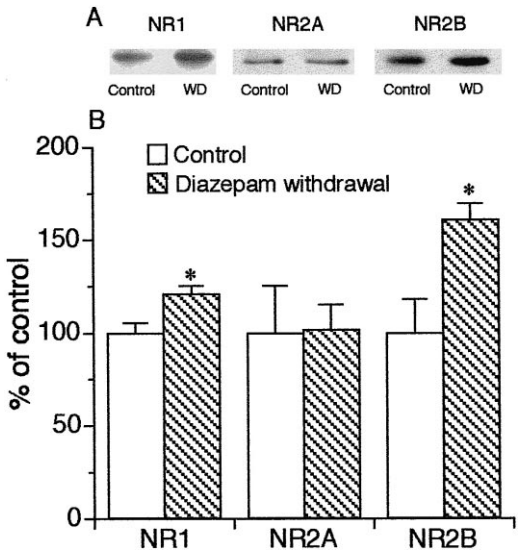

Fig. 1. Protein levels of the NR1, NR2A and NR2B subunits in the cerebral cortex during diazepam withdrawal. (A) Representative Western blot of NMDA receptor NR1, NR2A and NR2B subunit proteins. The molecular weights of the NR1, NR2A and NR2B subunits were 112, 174 and 164 kDa, respectively. (B) Changes in the protein levels of the NR1, NR2A and NR2B subunits in the cerebral cortex from control and diazepam-withdrawn rats. Ordinate: percentage of the respective value in control rats. Each column represents the mean with S.E.M. of 3–4 samples. $^{*}P < 0.05$ versus control group, Student's *t*-test.

withdrawn rats on the same blot were expressed as a percentage of those for control rats.

The levels of the NR1 (112 kDa) and NR2B (164 kDa) subunit proteins were significantly increased (120.8 and 160.7%, respectively) in diazepam-withdrawn rats (Fig. 1), while there was no change in the level of the NR2A subunit protein (174 kDa) between control and diazepam-withdrawn rats.

The present study demonstrated for the first time that the protein levels of the NR1 and NR2B, but not NR2A, subunits were increased during diazepam withdrawal. The drug-admixed food (DAF) method which was used to evaluate the physical dependence on diazepam in the present study is currently the only known method in which severe withdrawal signs spontaneously appear with the discontinuation of chronic diazepam treatment (Suzuki et al., 1992; Tsuda et al., 1997b). Since cerebrocortical tissue was removed from diazepam-withdrawn rats at 42–45 h after the termination of diazepam treatment when maximal withdrawal signs were observed, both upregulation of the NR1 and NR2B subunit proteins in the cerebral cortex and diazepam withdrawal signs occur simultaneously. We recently demonstrated that [^{3}H]dizocilpine binding sites in the cerebral cortex were upregulated during diazepam withdrawal. Several molecular pharmacological studies have suggested that the blocking effect of dizocilpine is more sensitive to recombinant NR1–NR2A and NR1–NR2B receptors than to recombinant NR1–NR2C and NR1–NR2D receptors (Laurie and Seeburg, 1994; Mori and Mishina, 1995). Therefore, an increase in the NR1 and NR2B subunit proteins may be responsible for the upregulation of [^{3}H]dizocilpine binding in the cerebral cortex. Our previous behavioral findings that diazepam withdrawal signs were suppressed by NMDA receptor antagonists 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 may trigger the appearance of diazepam withdrawal signs with the discontinuation of chronic diazepam treatment.

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