ELSEVIER

European Journal of Pharmacology 344 (1998) 27–30

ejp

Short communication

Role of nitric oxide in the hypersusceptibility to pentylenetetrazole-induced seizure in diazepam-withdrawn mice

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Received 16 October 1997; revised 23 December 1997; accepted 6 January 1998

Abstract

The decrease in the seizure threshold for pentylenetetrazole in diazepam-withdrawn mice was not significantly affected by L-arginine (50 and 100 μg/mouse, i.c.v.), which did have an antiseizure effect in chronically vehicle-treated mice. Sodium nitroprusside (25 and 50 μg/mouse, i.c.v.) increased the seizure threshold for pentylenetetrazole in both diazepam-withdrawn mice and chronically vehicle-treated mice. In addition, the antiseizure effect of L-arginine was blocked by the nitric oxide (NO) synthase inhibitor, *N*-nitro-L-arginine (NOARG) and the NO scavenger, hemoglobin, while the effect of sodium nitroprusside was inhibited by hemoglobin, but not by NOARG, indicating that the antiseizure effect of L-arginine, but not that of sodium nitroprusside, is mediated by NO production resulting from the activation of NO synthase. Therefore, a decrease in the NO production via NO synthase may be involved in the hypersusceptibility to pentylenetetrazole during diazepam withdrawal. © 1998 Elsevier Science B.V.

Keywords: L-Arginine; Sodium nitroprusside; Nitric oxide (NO) system; Diazepam withdrawal; Pentylenetetrazole-induced seizure (mouse)

1. Introduction

Nitric oxide (NO), formed from L-arginine by the enzyme NO synthase, is a short-lived, highly reactive messenger molecule that is synthesized in several tissues including the brain. NO is known to be involved in pathophysiological processes such as neurotoxicity and kindling (Rondouin et al., 1992; Garthwaite and Boulton, 1995). With regard to seizure, Buisson et al. (1993) have demonstrated that *N*-methyl-D-aspartate (NMDA)-induced seizure is potentiated by pretreatment with the NO synthase inhibitor, *N*-nitro-L-arginine methyl ester (L-NAME), in mice. Kainate-induced seizure is inhibited and enhanced by L-arginine (NO precursor) and L-NAME, respectively (Przegalinski et al., 1994). We previously reported that the NO synthase inhibitor, *N*-nitro-L-arginine (NOARG), aggravated the seizure induced by inhibitory agents of the $GABA_A$ receptor, pentylenetetrazole and DMCM (methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate), in mice (Tsuda et al., 1997c). This evidence suggests that NO plays a role as an endogenous antiseizure substrate in the central nervous system (CNS). Recent findings have shown that NO exerts a negative modulatory action against NMDA receptor function (Lei et al., 1992; Manzoni et al., 1992). Furthermore, we previously reported that the NMDA receptor was involved in pentylenetetrazole- and DMCM-induced seizures in mice (Tsuda et al., 1997a). Therefore, the inhibitory effect of NO on pentylenetetrazole- and DMCM-induced seizures may be due to its negative modulatory action against NMDA receptors.

It is well known that chronic administration of benzodiazepines causes physical dependence. Withdrawal signs after the discontinuation of chronic treatment with benzodiazepines are characterized by a loss of body weight, spontaneous seizure, increased muscle tone and a decrease in the seizure threshold for convulsants (such as pentylenetetrazole) (Woods et al., 1992). Recently, we demonstrated that the seizure threshold for pentylenetetrazole was markedly decreased during diazepam withdrawal: this hypersusceptibility to pentylenetetrazole-induced seizure was abolished by NMDA receptor antagonists (Tsuda et al., 1997b). It has been suggested that activation of the NMDA receptor may be responsible for the expression of diazepam withdrawal signs. Since NO plays a role as a negative modulator of NMDA receptor function, the

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role of NO in the expression of diazepam withdrawal signs was of interest. Therefore, we investigated the effects of two NO-producing agents, L-arginine and sodium nitroprusside, on the hypersusceptibility to 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

The threshold for pentylenetetrazole-induced seizure was examined as described previously (Tsuda et al., 1997b). 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. The mice were injected i.c.v. with L-arginine (50 and 100 μg/mouse), D-arginine (100 μg/mouse) or sodium nitroprusside (25 and 50 μg/mouse) 15 min before pentylenetetrazole infusion. Mice were injected with hemoglobin (100 μg/mouse, i.c.v.) and *N*-nitro-L-arginine (NOARG: 4 mg/kg, i.p.) 15 min and 1 h before pentylenetetrazole infusion. The injection volume for i.c.v. injection was 5 μl according to the methods of Haley and McCormick (1957) and Funada et al. (1993).

2.4. Drugs

Diazepam (Profarma, Italy) was suspended in vehicle consisting of 9% Tween 80 (Kishida Chemical, Osaka, Japan) in saline. Pentylenetetrazole, L-arginine, D-arginine, sodium nitroprusside, hemoglobin (Sigma Chemical, St. Louis, USA) and *N*-nitro-L-arginine (Research Biochemicals, MA, USA) were dissolved in saline.

2.5. Statistical analysis

The seizure threshold was evaluated statistically using the non-parametric Wilcoxon test.

3. Results

The seizure threshold for pentylenetetrazole was significantly increased by pretreatment with L-arginine (100 μg/mouse, i.c.v.) ($P < 0.01$; Fig. 1) in naive mice, while D-arginine (100 μg/mouse, i.c.v.), an inactive enantiomer of L-arginine, had no effect (data not shown). The antiseizure effect of L-arginine was significantly suppressed by NOARG (4 mg/kg, i.p.) or hemoglobin (100 μg/mouse, i.c.v.) ($P < 0.05$). Treatment with sodium nitroprusside (50 μg/mouse, i.c.v.) significantly increased the threshold for pentylenetetrazole-induced seizure ($P < 0.05$; Fig. 1). The antiseizure effect of sodium nitroprusside was suppressed by co-administration of hemoglobin (100 μg/mouse, i.c.v.) ($P < 0.05$). However, NOARG (4 mg/kg, i.p.) did not affect the antiseizure effect of sodium nitroprusside. NOARG (4 mg/kg, i.p.) or hemoglobin (100 μg/mouse, i.c.v.) had no effect on the threshold for pentylenetetrazole-induced seizure (data not shown).

Withdrawal from chronic treatment with diazepam significantly decreased the seizure threshold for pentylenetetrazole by approximately 26–28% ($P < 0.01$; Fig. 2). This decrease in the seizure threshold for pentylenetetrazole during diazepam withdrawal was not modified by pretreat-

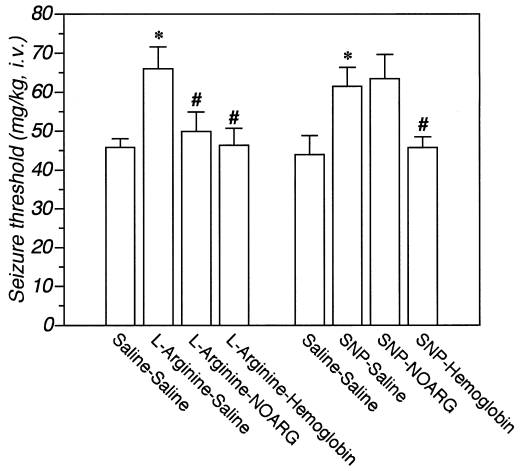

Fig. 1. Antiseizure effects of L-arginine and sodium nitroprusside on the threshold for pentylenetetrazole-induced seizure, and the effects of *N*-nitro-L-arginine (NOARG) and hemoglobin on the antiseizure activities produced by L-arginine and sodium nitroprusside in mice. Mice were injected with L-arginine (100 μg/mouse, i.c.v.) and sodium nitroprusside (SNP: 50 μg/mouse, i.c.v.) 15 min before i.v. infusion with pentylenetetrazole. NOARG (4 mg/kg, i.p.) and hemoglobin (100 μg/mouse, i.c.v.) were injected 60 and 15 min before i.v. infusion of pentylenetetrazole, respectively. Ordinate: seizure threshold for pentylenetetrazole (mg/kg, i.v.). Each column represents the mean with S.E.M. for 7–15 mice. * $P < 0.05$ vs. each saline–saline group. # $P < 0.05$ vs. L-arginine- or sodium nitroprusside-saline group.

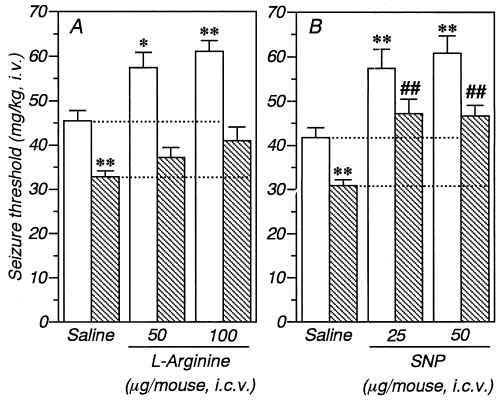

Fig. 2. Effects of L-arginine (A) and sodium nitroprusside (B) on the decrease in the seizure threshold for pentylenetetrazole during diazepam withdrawal in mice. Mice were injected with L-arginine (50 and 100 μg/mouse, i.c.v.) and sodium nitroprusside (SNP: 25 and 50 μg/mouse, i.c.v.) 15 min before i.v. infusion with pentylenetetrazole. Chronic treatment: vehicle (open column) or diazepam (hatched column). Ordinate: seizure threshold for pentylenetetrazole (mg/kg, i.v.). Each column represents the mean with S.E.M. for 9–14 mice. $*\ P < 0.05$, $**\ P < 0.01$ vs. pretreatment with saline in the chronic vehicle group. $\#\#P < 0.01$ vs. pretreatment with saline in the chronic diazepam group.

ment with L-arginine (50 and 100 μg/mouse, i.c.v.) which produced a significant increase in the threshold for pentylenetetrazole-induced seizure in chronically vehicle-treated mice ($P < 0.01$; Fig. 2A). Pretreatment with sodium nitroprusside (25 and 50 μg/mouse, i.c.v.) significantly reduced the decrease in the seizure threshold for pentylenetetrazole in diazepam-withdrawn mice ($P < 0.01$; Fig. 2B) and increased the seizure threshold for pentylenetetrazole in vehicle-treated mice ($P < 0.01$).

4. Discussion

As the first step in the present study, we demonstrated that i.c.v. administration of the NO precursor, L-arginine, or the NO donor, sodium nitroprusside, has an antiseizure effect against pentylenetetrazole-induced seizure in mice. This finding is supported by our previous report that pentylenetetrazole-induced seizure is aggravated by NO synthase inhibitors (Tsuda et al., 1997c), and confirm the notion that NO is an endogenous antiseizure substrate in the brain. However, it is reported that pentylenetetrazole-induced seizure was not affected by the NO synthase inhibitor (Przegalinski et al., 1994; Tutka et al., 1996). This discrepancy may be due to differences in strain of mouse (ddY strain vs. Swiss strain) and the approach used to measure seizure (seizure threshold vs. seizure incidence). Pretreatment with NOARG, an inhibitor of NO synthase that inhibits the production of NO via NO synthase, abolished the antiseizure effect of L-arginine, but not of sodium nitroprusside. On the other hand, hemoglobin, a NO scavenger that binds released NO, suppressed the antiseizure effects of both L-arginine and sodium nitroprusside. These results suggest that the antiseizure effect of L-arginine may be mediated by NO generated as a result of the activation of NO synthase, while the effect of sodium nitroprusside may be associated with NO liberated non-enzymatically.

The hypersusceptibility to pentylenetetrazole-induced seizure during diazepam withdrawal is consistent with previous findings (Tsuda et al., 1997b), which indicated withdrawal excitability in response to physical dependence on diazepam. The increase in seizure susceptibility to pentylenetetrazole during diazepam withdrawal was not significantly modified by L-arginine (50 and 100 μg/mouse, i.c.v.), which had a significant suppressive effect on pentylenetetrazole-induced seizure in chronically vehicle-treated mice. This result indicates that the antiseizure effect of L-arginine is reduced during diazepam withdrawal. However, another NO-producing agent, sodium nitroprusside, which, unlike L-arginine, produces NO without the activation of NO synthase, produced an increase in the threshold for pentylenetetrazole-induced seizure in both diazepam-withdrawn mice and vehicle-treated control mice. The subsensitivity to the antiseizure effect of L-arginine, but not that of sodium nitroprusside, in diazepam-withdrawn mice leads to the hypothesis that the capacity for NO production from L-arginine via NO synthase may be reduced during diazepam withdrawal. These findings are interesting in the light of evidence that NO may play a role as an endogenous antiseizure substrate in the CNS (Buisson et al., 1993; Przegalinski et al., 1994; Tsuda et al., 1997c). A relationship between the hypersusceptibility to seizure and the reduction of NO production via NO synthase has been described previously. The number of NADPH diaphorase-positive cells in the hippocampus is significantly lower in the EL mouse (an inbred mutant strain of ddY mouse which is hypersusceptible to seizure) than in the ddY mouse (Nagatomo et al., 1996). NO synthase activity in a seizure-sensitive group of Mongolian gerbils was found to be significantly lower than that in a seizure-resistant group (Iwahashi et al., 1995). In our previous study, the hypersusceptibility to pentylenetetrazole-induced seizure in diazepam-withdrawn mice was mimicked by treatment with NO synthase inhibitors in naive mice (Tsuda et al., 1997c), suggesting that the decrease in the capacity for NO production via NO synthase may be responsible for the expression of hypersusceptibility to pentylenetetrazole-induced seizure during diazepam withdrawal.

The recent finding that NO regulates NMDA receptor function negatively through an interaction with the redox modulatory site (Lei et al., 1992; Manzoni et al., 1992) leads to the hypothesis that the reduction of the L-arginine response may result in the enhancement of NMDA receptor function. NMDA-induced seizure is potentiated by pretreatment with a NO synthase inhibitor (Buisson et al., 1993). Considering the previous finding that NMDA-induced seizure is enhanced in diazepam-withdrawn mice (Steppuhn and Turski, 1993; Tsuda et al., 1998), the

reduction of NO levels may cause functional changes in the NMDA receptor which in turn accelerate signs of diazepam withdrawal.

5. Conclusion

The present study demonstrated that (1) the antiseizure effect of L-arginine is mediated by NO production from L-arginine via NO synthase while the effect of sodium nitroprusside is associated with NO liberated non-enzymatically, and (2) the decrease in NO production via NO synthase may be involved in the hypersusceptibility to pentylenetetrazole during diazepam withdrawal.

Acknowledgements

This work was supported in part by a Grant-in-Aid from The Tokyo Biochemical Research Foundation and a Research Grant for Nervous and Mental Disorders from the Ministry of Health and Welfare to T.S. We wish to thank Ms. Sachiko Komiya and Ms. Miho Soma for their expert technical assistance.

References

Buisson, A., Lakhmeche, N., Verrecchia, C., Plotkine, M., Boulu, R.G., 1993. Nitric oxide: an endogenous anticonvulsant substance. NeuroReport 4, 444–446.

Funada, M., Suzuki, T., Narita, M., Misawa, M., Nagase, H., 1993. Modification of morphine-induced locomotor activity by pertussis toxin: biochemical and behavioral studies in mice. Brain Res. 619, 163–172.

Garthwaite, J., Boulton, C.L., 1995. Nitric oxide signaling in the central nervous system. Annu. Rev. Physiol. 57, 683–706.

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.

Iwahashi, K., Yoneyama, H., Miyatake, R., Ichikawa, Y., Hosokawa, K., 1995. NOS activity in mutant rodents susceptible for epilepsy or convulsion. J. Jpn. Epilepsy Soc. 13, 21–23, in Japanese with English Abstract.

Lei, S.Z., Pan, Z.-H., Aggarwal, S.K., Chen, H.-S.V., Hartman, J., Sucher, N.J., Lipton, S.A., 1992. Effect of nitric oxide production of the redox modulatory site of the NMDA receptor-channel complex. Neuron 8, 1087–1099.

Manzoni, O., Prezeau, L., Marin, P., Deshager, S., Bockaert, J., Fagni, L., 1992. Nitric oxide-induced blockade of NMDA receptors. Neuron 8, 653–662.

Nagatomo, I., Akasaki, Y., Kuchiiwa, S., Nakagawa, S., Takigawa, M., 1996. Sparse distribution of NADPH diaphorase neurons in the hippocampal formation of the inbred mutant strain EL mouse. Brain Res. 730, 223–226.

Przegalinski, E., Baran, L., Siwanowicz, J., 1994. The role of nitric oxide in the kainate-induced seizure in mice. Neurosci. Lett. 170, 74–76.

Rondouin, G., Lerner-Natoli, M., Manzoni, O., Lafon-Cazal, M., Bockaert, J., 1992. A nitric oxide (NO) synthase inhibitor accelerates amygdala kindling. NeuroReport 3, 805–808.

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

Tsuda, M., Suzuki, T., Misawa, M., 1997a. Role of the NMDA receptor complex in DMCM-induced seizure in mice. NeuroReport 8, 603–606.

Tsuda, M., Suzuki, T., Misawa, M., 1997b. 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., 1997c. Aggravation of DMCM-induced seizure by nitric oxide synthase inhibitors in mice. Life Sci. 60, 339–343.

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

Tutka, P., Klonowski, P., Dzieciuch, J., Kleinrok, Z., Czuczwar, S.J., 1996. N^G-Nitro-L-arginine differentially affects glutamate- or kainate-induced seizure. NeuroReport 7, 1605–1608.

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