The N-Methyl-D-Aspartate (NMDA) Receptor Antagonist Dizocilpine (MK-801) Suppresses Enflurane-Induced Opisthotonus in Mice

Hisao KOMATSU, Junko NOGAYA*, Daisuke ANABUKI* and Kenji OGLI*

We determined whether enflurane-induced opisthotonus in ddN mice is mediated by N-methyl-D-aspartate (NMDA) receptor using NMDA receptor antagonists dizocilpine (MK-801) and ketamine. Animals were given intraperitoneal injections of 0.2 ml saline (control), 2.5 or 5.0 mg·kg^{-1} dizocilpine in saline, or 20 or 40 mg·kg^{-1} ketamine in saline 20 min prior to exposure to 2.0% enflurane. Incidence of opisthotonus measured during exposure to enflurane for 20 min was 49% (n=51) in saline (control) group, 6.7 ($P < 0.01$ vs control, n=30) and 15.0% ($P < 0.01$, n=40) in 2.5 and 5.0 mg·kg^{-1} dizocilpine group, respectively, and 43.9 (NS, n=41) and 40.0% (NS, n=40) in 20 and 40 mg·kg^{-1} ketamine group, respectively. These results strongly suggest that enflurane-induced opisthotonus is mediated by NMDA receptor. Ketamine failed to suppress significantly due to possibly small dosages. Further, dizocilpine itself produced severe seizures during pre-enflurane period (30.0 and 40.0% in 2.5 and 5.0 mg·kg^{-1}, respectively), which may be a novel finding. (Key words: enflurane-induced opisthotonus, NMDA receptor, dizocilpine (MK-801))

(Komatsu H, Nogaya J, Anabuki D, et al.: The N-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine (MK-801) suppresses enflurane-induced opisthotonus in mice. J Anesth 7: 519–522, 1993)

We previously reported that volatile anesthetics produce various incidences of opisthotonus[1,2] which is thought to be a sign of central nervous system (CNS) stimulation[3]. The order of incidence of opisthotonus was as follows: sevoflurane > isoflurane > enflurane > methoxyflurane > halothane[2]. The excitatory amino acids, glutamate and aspartate, are found throughout the mammalian CNS where they are believed to subserve excitatory synaptic transmission[4]. The receptors mediating their actions are generally divided into the three major subtypes that have been pharmacologically and electrophysiologically identified on the basis of agonists which selectively activate them: N-methyl-D-aspartate (NMDA), kinate, and quisqualate[5,6]. Antagonists of the NMDA receptor subtype have been shown to possess strong

Department of Anesthesiology, National Kagawa Children's Hospital, Kagawa, Japan

**Department of Anesthesiology and Emergency Medicine, Kagawa Medical School, Kagawa, Japan*

Address reprint requests to Dr. Komatsu: Department of Anesthesiology, National Kagawa Children's Hospital, 2603, Zentsuji-cho, Zentsuji, Kagawa, 765 Japan

Table 1. Effects of intraperitoneal dizocilpine and ketamine on incidence of enflurane-induced opisthotonus in ddN mice

	n	seizures during per-enflurane period (%)	enflurane-induced opisthotonus (%)
saline	51	0	49.0
dizocilpine 2.5 mg·kg^{-1}	30	30.0	6.7*
dizocilpine 5.0 mg·kg^{-1}	40	40.0	15.0*
ketamine 20 mg·kg^{-1}	41	0	43.9
ketamine 40 mg·kg^{-1}	40	0	40.0

*Significant difference compared with saline group, $P < 0.01$

anticonvulsant properties in various acute chemical[7,8] and chronic kindling[9] seizure models. Dizocilpine (MK-801) is a selective, non-competitive antagonist of excitatory amino acid transmitters at the NMDA receptor site[10,11] and ketamine also selectively reduces excitation of mammalian and lamprey neurones by NMDA[12,13]. The present experiment was designed to investigate the role of NMDA receptor in the development of enflurane-induced opisthotonus using dizocilpine and ketamine.

Young adult (10 ± 2 wk old) male ddN mice obtained from Kagawa Medical School Experimental Animal Laboratory served as subjects. Animals were housed with male littermates in plastic cages with wood chip bedding in a colony room maintained on a 12h/12h light – dark schedule and a constant room temperature of 24°C. Animals were permitted free access to food and water *ad libitum*.

Twenty minutes prior to measurement of enflurane-induced opisthotonus, animals were given intraperitoneal injections of saline (as a control), 2.5 or 5.0 mg·kg^{-1} dizocilpine, or 20 or 40 mg·kg^{-1} ketamine. For injections of dizocilpine and ketamine, the agents were dissolved in saline solution at concentrations of 0.6 mg·ml^{-1} or 1.2 mg·kg^{-1} and 3.0 or 6.0 mg·ml^{-1}, respectively. Thus, each mouse was injected approximately 0.2 ml solution intraperitoneally. For twenty min after injection, mice were observed for opisthotonus or seizure. No animal showed any behavioral changes in the saline group. In most animals both in the dizocilpine and ketamine groups, reeling, lateral head-nodding, calming down, and circling were observed. In the dizocilpine groups, severe, but short duration seizures were observed in 30.0 and 40.0% of animals in 0.25 and 0.5 mg·kg^{-1} dizocilpine groups, respectively. The seizure was observed 5–10 min after injection and included a stormy wild running, struggling and jumping for at most 3 seconds. No seizures were seen in the ketamine groups. No opisthotonuses and seizures were exhibited at the beginning of exposure to enflurane.

Animals were anesthetized with 2.0% enflurane in air (5 l·min^{-1}) for 20 min in a 171 plastic chamber and were observed for opisthotonus by a single observer who has no knowledge

of the agents. Opisthotonus was defined as the case when both head and tail bent backward about 15 degrees or more from the holizontal plane[2]. We had adopted 30 degrees as a criterion of opisthotonus[1], however 15 degrees was thought to be more preferable than 30 degrees because it is more sensitive to detect opisthotonus and is still enough to recognize opisthotonus.

Results are shown in table 1. Dosages of 2.5 and 5.0 $mg \cdot kg^{-1}$ of dizocilpine strongly suppressed enflurane-induced opisthotonus ($P < 0.01$). This clearly suggests that the opisthotonus is mediated by the NMDA receptor.

In contrast, ketamine that is believed to have NMDA-antagonistic properties showed just an inhibitory tendency of the opisthotonus. This may be due to relatively lower dosages of ketamine than those of dizocilpine. Gilbert demonstrated that dizocilpine produced a dramatic retardation in the development of amygdala kindling, but not a dosage level that was without behavioral side effects[9]. In our present study, ketamine (20 and 40 $mg \cdot kg^{-1}$) produced only fine tremor and reeling, while dizocilpine (2.5 and 5.0 $mg \cdot kg^{-1}$) exhibited even strong seizures including jumping and struggling. There was no correlation between dizocilpine-induced seizure and enflurane-induced opisthotonus. The reason of the seizure is unknown, but it is possible that dizocilpine may disrupt the balance between inhibitory (e.g. GABA) and excitatory (e.g. NMDA, acetylcholine) systems within other brain regions than amygdala[9]. Yamamura et al. indicated that 50% block of the responses induced by NMDA took place at ketamine concentrations between 10 and 20 μM, and all of the responses were abolished at the concentrations higher than 40–50 μM in *in vitro* preparations in lamprey CNS[13]. Although we did not determine the plasma ketamine concentration, it may be less than 40 μM given that plasma concentrations of ketamine are reported to be approximately between 1 and 6 μM in humans[14] and do not exceed 40 μM in animals[15] when 20–40 $mg \cdot kg^{-1}$ was given from various routes. Different potencies (dizocilpine > ketamine) in antagonizing the excitatory actions of NMDA[10,16] may also relate to the current results.

Acknowledgement: The authors thank Nobuko Kimura for assitance in the experiment.

(Received Oct. 23, 1992, accepted for publication Feb. 27, 1993)

References

1. Komatsu H, Ogli K: Opisthotonus during exposure to isoflurane, enflurane, and halothane in mice. Anesthesiology 67: 771–774, 1987
2. Komatsu H, Ohara T, Nogaya J, et al: The effects of age and anesthetic solubility in anesthetic-induced opisthotonus in mice. J Anesth 5:228–232, 1991
3. Ruch TC: Brain stem control of posture and orientation in space, The brain and neural function, Physiology and Biophysics, Vol. 1. Edited by Ruch TC, Patton HD. Philadelphia, WB Saunders, 1979, pp 14–52
4. Watkins JC, Evans RH: Excitatory amino acid neurotransmitters. Ann Rev Pharmacol Toxicol 21:165–204, 1981
5. Davis J, Evans RH, Jones AW, et al: Differential activation and blockade of excitatory amino acid receptors in mammalian and amphibian CNS. Comp Biochem Physiol 72C:211–224, 1982
6. McLennan H: Receptors for excitatory amino acids in mammalian central nervous system. Prog Neurobiol 20:251–271, 1984
7. Croucher MJ, Collins JF, Meldrum BF: Anticonvulsant action of excitatory amino acid antagonists. Science 216:899–901, 1982
8. Avioli M, Olivier A: Bursting in human epileptogenic neocortex is de-

pressed by an N-methyl-D-aspartate antagonist. Neurosci Lett 76:249–254, 1987

9. Gilbert ME: The NMDA-receptor antagonist, MK-801, suppresses limbic kindlings and kindled seizures. Brain Res 463:90–99, 1989
10. Wong EHF, Kemp JA, Priestley T, et al: The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. Proc Natl Acad Sci USA 83:7104–7108, 1986
11. Foster AC, Wong EHF: The novel anticonvulsant MK-801 binds to the activated state of the N-methyl-D-aspartate receptor in rat brain. Br J Pharmacol 91:403–409, 1987
12. Anis NA, Berry SC, Benton NR, et al: The dissociative anesthetics ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-D-aspartate. Br J Pharmacol 79:565–575, 1983
13. Yamamura T, Harada K, Okamura A, et al: Is the site of action of ketamine anesthesia the N-methyl-D-aspartate receptor? Anesthesiology 72:704–710, 1990
14. Wieber J, Gugler R, Hengstmann JH, et al: Pharmacokinetics of ketamine in man. Anaesthetist 24:260–263, 1975
15. Oswald RL: Injection anesthesia for experimental studies in fish. Comp Biochem Physiol 60C:19–26, 1978
16. Lodge D, Anis NA: Effects of phencyclidine on excitatory amino acid activation of spinal interneurones in the cat. Eur J Pharmacol 77:203–204, 1982