Intrathecal administration of N-methyl-D-aspartate receptor antagonist reduces the minimum alveolar anaesthetic concentration of isoflurane in rats

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Summary
We have studied the effect of intrathecal administration of N-methyl-D-aspartate (NMDA) receptor antagonists on the minimum alveolar anaesthetic concentration (MAC) of isoflurane in rats. In Wistar rats fitted with indwelling intrathecal catheters, we determined the MAC of isoflurane after administration of a competitive NMDA receptor antagonist APV (0.01, 0.1, 1.0, 10, 30 µg), a non-competitive NMDA receptor antagonist, MK801 (0.1, 1.0, 10, 30 µg), NMDA (0.01, 0.1, 1.0, 10, 30 µg) and saline. APV at all doses except 0.01 µg decreased MAC by 17.1–32 % (P < 0.001 and P < 0.0001). Although MK801 at 10 and 30 µg reduced MAC by 24.3–31.7 % (P < 0.001 and P < 0.0001), lower doses did not affect MAC. Intrathecal administration of NMDA reversed these decreases in MAC, but not to control values with APV 10 and 30 µg and MK801 30 µg. We suspect that NMDA and NMDA receptor antagonists play important roles in the spinal cord in determining the MAC of isoflurane. (Br. J. Anaesth. 1995; 75: 636–638)

Key words
Anaesthetics volatile, isoflurane. Potency, anaesthetic, MAC. Receptors, amino acid. Antagonists NMDA receptor. Rat.

Despite the widespread use of inhalation anaesthetics, the mechanisms and sites of action of most agents remain unknown. Many experiments have been directed towards the assessment of anaesthetic effects on the cerebral cortex. However, we know that inhalation anaesthetics exert significant effects elsewhere, such as on the spinal cord [1]. It is not known if the site of anaesthetic action is the cortex or spinal cord. In a recent study in the rat, cortical structures were removed without significantly altering the minimum alveolar anaesthetic concentration (MAC) of the inhalation anaesthetics [2], suggesting that the relevant sites for MAC determination are the brain stem and the spinal cord. The potency of inhalation anaesthetics is defined as the MAC required to prevent movement in 50 % of test subjects. A state of anaesthesia is, therefore, defined in terms of the motor response to a noxious stimulus, usually a flexion withdrawal reflex, mediated at the spinal cord. A role for excitatory amino acids (EAA) in nociception is suggested by the fact that noxious stimulation causes release of glutamate and aspartate in the spinal cord dorsal horn. Intrathecal administration of N-methyl-D-aspartate (NMDA) produces a rapid, transient, dose-dependent hyperalgesia. This hyperalgesia is blocked reversibly by prior treatment with the selective NMDA receptor antagonist, APV (2-amino-5-phosphonopentanoic acid) [3]. It has also been shown that the release of peptide and of an amino acid neurotransmitter from primary afferents is not abolished by inhalation anaesthetics. A recent study has shown that the systemic administration of NMDA receptor antagonists reduces the MAC of isoflurane [4].

The purpose of this study was to investigate in rats the effect of intrathecal administration of an NMDA receptor antagonist on the MAC of isoflurane.

Methods and results
This investigation was approved by the Animal Care Committee of Gunma University. Male Wistar rats (280–320 g) were fitted with indwelling intrathecal catheters by methods described previously [5]. In brief, the rat was mounted in a conventional stereotaxic instrument under halothane anaesthesia. Chronic intrathecal catheters were placed by passing a PE10 catheter through an incision in the atlanto–occipital membrane to a position 8 cm caudal to the cisterna at the level of the lumbar enlargement. The catheter was externalized on the top of the skull and the wound was closed with 3-0 silk sutures. Preliminary trials with Evans Blue dye added to the injected solution had confirmed the lumbar enlargement. Rats with postoperative neurological deficits were killed promptly with an overdose of barbiturate. MAC was measured 4–9 days after intrathecal implantation of the catheter.

The agents studied were: MK801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate; Research Biochemical Inc, MA, USA); APV (2-amino-5-phosphonopentanoic acid; Sigma); and NMDA (N-methyl-D-aspartate; Sigma). All drugs were dissolved in normal saline, so that the final dose was administered in a volume of...
The temperature was maintained at 36.5–37.5°C by use of a heating pad and thermometer system (CMA150, Microdialysis AB, Stockholm, Sweden). The extradural heating pad was removed at the end of the experiments. A polyethylene catheter (PE50) was inserted through the tracheotomy, and the tip of the PE50 was inserted through the wall of the ventilatory tubing, and the tip of the sampling tube was positioned distal to the tracheotomy, thus allowing for repeated sampling of end-tidal gases. For arterial pressure monitoring and blood sampling, one femoral artery was catheterized with a 14-gauge polyethylene catheter (PE50) inserted through the tracheotomy. Rats breathed spontaneously.

Ten µl of 0.1, 1.0, 10 or 30 µg of control saline was infused intrathecally. Figure 1 shows the changes in MAC of isoflurane caused by intrathecal administration of MK801 and NMDA. Significant difference between saline and APV (P < 0.001); b = significant difference between saline and APV 10, 30 µg (P < 0.0001); c = significant difference between saline and NMDA (P < 0.05); d = significant difference between saline and NMDA (P < 0.01). Right: Mean (SEM) changes in MAC of isoflurane caused by intrathecal administration of MK801 and NMDA. a = significant difference between saline and APV 0.1 µg, b = APV 1.0 µg, c = APV 10 µg, d = APV 30 µg. a = significant difference between saline and MK801 30 µg (P < 0.0001); b = significant difference between saline and MK801 30 µg (P < 0.0001); c = significant difference between MK801 0.1, 1.0 µg and MK801 10 µg (P < 0.01); d = significant difference between MK801 0.1, 1.0 µg and MK801 30 µg (P < 0.001); e = significant difference between saline and NMDA (P < 0.01).

Administration of NMDA receptor antagonists decreased the MAC of isoflurane, but this decrease was reversed by administration of NMDA. It has been suggested that the NMDA receptor is related to the MAC of inhalation anaesthetics [5].

NMDA antagonists are divided into two groups, a competitive, selective type and a non-competitive, non-selective type. The competitive NMDA receptor antagonist, APV, directly blocks the glutamate recognition site. MK801, a non-competitive antagonist, directly blocks the gluta-
Antagonist, binds its recognition site within the NMDA receptor-activated membrane channel and thus impedes cation flow through the channel. It has been shown that intrathecal administration of APV causes significant antinociception in the mouse formalin model of tonic chemogenic nociception [6]. APV has also been shown to be antinociceptive as evidenced by its ability to attenuate non-reflex responses to noxious electrical and pressure stimuli in the rat, and also by its inhibition of the hyperalgesic effect of intrathecal administration of NMDA in mouse thermal tests [4]. Ketamine, a non-competitive NMDA receptor antagonist, probably produces analgesic effects by disrupting sensory input in the spinal cord and thalamus. MK801 also has an antinociceptive effect, and an MK801 binding site has been reported in the spinal cord. APV decreased MAC at 0.1, 1.0, 10 and 30 µg, and this decrease was reversed by administration of NMDA. Although the reversal of NMDA effects to control values was incomplete at APV 10 and 30 µg and MK801 30 µg, this is considered to result from motor nerve dysfunction. Cahusac and colleagues [6] showed that intrathecal administration of APV 12–48 µg produced some locomotor depression in rats. Motor nerve dysfunction produced by APV could reflect an increase in extensor tone produced by a reduction in postsynaptic inhibition of motor-neurones, following antagonism of synaptic excitation of Renshaw cells or other interneurones.

Recent evidence suggests that C-fibre excitatory amino acids may facilitate plastic changes in spinal cord dorsal horn neurones that are induced by noxious stimulation. There is evidence that central sensitization and wind-up are dependent on NMDA receptor activation and that NMDA receptor antagonists may prevent behaviours indicative of pain. The only NMDA receptor antagonist licensed for use in patients is the dissociative anaesthetic ketamine, which is unpleasant for most patients when given systemically. Nevertheless, extradural ketamine produces potent postoperative analgesia. It does suggest new possibilities of treatment if NMDA receptor antagonists without unacceptable side effects are developed and if they can be administered via the extradural route with no systemic actions. NMDA receptor antagonists may reduce the requirement for inhalation anaesthetics and decrease postoperative pain.

References