Effects of xenon on acetylcholine release in the rat cerebral cortex \textit{in vivo}

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\textbf{Background.} We have reported previously the effects of several anaesthetics on cholinergic activity in the central nervous system (CNS). In this study, we report the effects of xenon on cholinergic cell activity.

\textbf{Methods.} Using \textit{in vivo} brain microdialysis, we measured acetylcholine (ACh) release in the rat cerebral cortex \textit{in vivo} during xenon anaesthesia.

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Xenon and ACh release

Results. Xenon induced an initial increase in ACh release, followed by a gradual decrease. The level of ACh release at 40 min of xenon administration was significantly higher than the control.

Conclusions. Xenon activates CNS cholinergic cell activity followed by development of acute tolerance.

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Xenon is an inert gas that has anaesthetic properties. It is non-explosive, non-toxic, reactive, and does not undergo biotransformation. Induction of and emergence from anaesthesia using xenon are rapid because of its low blood-gas partition coefficient (0.14).1 Despite the advantages, it has not been used in clinical practice because of its expense. Nitrous oxide, which is the most popular anaesthetic gas, induces megaloblastic bone marrow changes2 and myeloneuropathy.3 It also disturbs the earth’s ozone layer.4 The disadvantages of nitrous oxide, combined with the recent trend toward low-flow or closed-circuit anaesthesia techniques, have caused anaesthetists to reconsider the use of xenon in place of nitrous oxide.

Based on the effects on the central nervous system (CNS) electrical activities, we have classified general anaesthetics into three categories: CNS depressant, CNS excitant, and epileptogenic,56 and reported that xenon7 and nitrous oxide7 are classified as excitatory. In contrast, isoflurane and sevoflurane are CNS depressant and epileptogenic,8 respectively.

We have reported that nitrous oxide enhances the release of acetylcholine (ACh), one of the major excitatory neurotransmitters in the rat cerebral cortex in vivo, whereas isoflurane and sevoflurane strongly suppressed ACh release.9 However, the effect of xenon has not been reported. In this study, we have examined the effects of xenon on ACh release in the rat cerebral cortex using an in vivo microdialysis technique.

Methods and results
The study was approved by the Animal Research Committee of Kyoto University Faculty of Medicine. Nine male Wistar rats, weighing 250–300 g, were used. They were housed, one per cage, under a 12-h light-dark cycle (lights on from 07:00 to 19:00) with free access to food and water. All microdialysis studies were started between 10:00 and 11:00. The animals were anaesthetized with sodium pentobarbitone (50 mg kg−1 i.p.), and placed on a stereotaxic frame. The skull was exposed through a midline incision of the scalp and a burr hole of 2-mm diameter was drilled. A guide cannula was implanted in the frontal cortex at A 3.0 mm and L 2.0 mm from the bregma, and was 2.0 mm in depth. The cannula was fixed to the skull with dental cement and a stainless steel screw. The location of the probe was confirmed by visual examination of the brain at the end of each experiment. Animals were allowed to recover for at least 1 day. On the day of the microdialysis experiment, the rat was anaesthetized in a clear plastic box (25×25×20 cm) in an atmosphere with 2% isoflurane and 75% nitrogen (3 litre min−1) in oxygen (1 litre min−1). The stylet was removed from the guide cannula and a microdialysis probe was inserted. Then isoflurane was stopped. The dialysis probe was connected to the perfusion pump and sample loop of an automated sample injector (model 10; EICOM, Kyoto, Japan) in a high-performance liquid chromatography (HPLC) system using polyethylene tubing. The internal standard, ethylhomocholine, was delivered by the perfusion pump into the perfusate tube proximal to the injection valve. The dialysis probe was perfused continuously with Ringer solution (147 mM Na+, 2.3 mM Ca2+, 4 mM K+, 156 mM Cl−) containing 10 μM physostigmine to prevent acetylcholinesterase from metabolizing ACh, at a rate of 2.0 μl min−1.

Perfusate samples obtained during the first 2 h after implantation of the probe were discarded to exclude the influence of isoflurane and probe insertion. The dialysates were then collected every 20 min in the sample loop of the automated sample injector, which was on line to an HPLC system. ACh was assayed using a HPLC-electrochemical detection system and a method described previously.9

After collecting three initial samples, 75% nitrogen was replaced with xenon (provided by Nippon Sanso Corp., Tokyo, Japan). After 5 min, the flow rate of xenon and oxygen was decreased to 300 and 100 ml min−1, respectively, to reduce xenon consumption. Four hours later, xenon was stopped and the control gas mixture was replaced for 2 h. Xenon and carbon dioxide concentrations in the box were monitored continuously using a xenon gas monitor (Anzai Sogyo, Tokyo), and oxygen concentration was monitored by an anaesthetic gas monitor (Type 1304; Brüel & Kjær, Denmark).

Basal ACh release was determined as the mean of the three initial collections. All data are expressed as percentage of the basal value and are mean (SEM). Statistical significance was assessed by analysis of variance with repeated measures and, when significant F values were obtained, Fisher’s protected least significant difference test.
was used for significant differences between treatment means. A probability level of $P<0.05$ was considered significant.

The concentration of xenon in the box reached the predicted value within 5 min, and carbon dioxide was kept under 1% throughout the experiment. The rats were quiet during the control period. They became alert and moved around while they inhaled xenon for approximately 5 min, then became quiet and seemed to sleep lightly. Within 3 min after cessation of xenon administration, the rats became alert, and then after approximately 10 min, they became quite as seen during the control period.

Baseline ACh release was stable, and the content was 3.7 (0.5) pmol 20 min$^{-1}$. ACh release was significantly increased to reach the maximal level of 184.0 (28.5)% in the first fraction (0–20 min, $P<0.01$), followed by a gradual decline (Fig. 1). After 40 min of exposure to xenon, there were no differences from the control values except for a transient increase in the sixth fraction (100–120 min).

**Comment**

The MAC of xenon in rats is 1.61 (0.17) atm; therefore, 75% xenon in atmospheric pressure corresponds to approximately 0.5 MAC. We demonstrated previously that 0.3 and 0.5 MAC of isoﬂurane and sevolurane signiﬁcantly suppressed ACh release. In contrast, 75% nitrous oxide (0.3 MAC) enhanced cortical ACh release in rats reaching a maximum level of 193.7 (20.0)% of the baseline value. Although we did not compare ACh release induced by xenon and nitrous oxide at equipotent concentrations, our results indicate that these two anaesthetics have similar effects on cortical ACh release. This notion is consistent with the results obtained in our previous electrophysiological study, which showed that xenon has a CNS stimulant action similar to nitrous oxide and belongs to the excitatory anaesthetics. Although cholinergic neurons have been postulated to have key roles in the maintenance of consciousness, activation of cortical cholinergic neurons induced by xenon or nitrous oxide does not necessarily correlate with an elevation in the level of consciousness.

The gradual decline in ACh release during exposure to xenon observed in the present study suggests the development of acute tolerance to the CNS action of xenon, and is consistent with our previous report of xenon effects on RMUA. Further studies are required to determine whether acute tolerance to other CNS actions of xenon develop in a similar fashion to nitrous oxide, such as the wake–sleep cycle and EEG, and whether changes in ACh release are the cause or result of tolerance.

In conclusion, xenon produces a stimulant effect on cholinergic neurons projecting to the cerebral cortex. Furthermore, acute tolerance to this stimulant effect develops.

**References**