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Does Xenon Anesthesia Inhibit Cholinesterases?

An In Vitro *Radiometric Assessment*

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PREVIOUSLY, we observed cholinergic effects of xenon in clinical use, namely decreased heart rate or increased salivary secretion.[1,2] A microdialysis technique showed transient increases in acetylcholine concentration in rat cerebral cortex when xenon was inhaled as an anesthetic,[3] while ether and cyclopropane have been known to inhibit acetylcholinesterase at clinically relevant concentrations.[4,5] Therefore, we hypothesize that xenon may affect the cholinergic nervous system by inhibiting acetylcholinesterase.

Materials and Methods

The Ethical Committee for Animal Research at the National Institute of Radiological Sciences (Chiba, Japan) approved this study. Male Wistar rats aged 10–12 weeks were decapitated during ether anesthesia, and cerebral cortex was homogenized and diluted with phosphate buffer, including sodium phosphate and potassium phosphate (0.1 M; pH, 7.4), for later use. Blood (5 ml) was also aspirated at the cervical stump with a heparinized syringe.

Inhibition of acetylcholinesterase was assessed by measuring the rate constant for hydrolysis of two substrates using a radiometric technique.[6] This enabled us to measure acetylcholinesterase activation while keeping the container airtight. *N*-[^{14}C]methylpiperidin-4-yl acetate and (R)-*N*-[^{14}C]methylpiperidin-3-yl butyrate are enzyme-specific, radioactive acetylcholine and butyrylcholine analogs, respectively.[6–8]

Two hundred microliters of brain homogenate (tenfold diluted) or blood (undiluted) was loaded in small glass tubes kept airtight with a septum cap. The gas phase in the tubes was filled either with 100% oxygen (control) or 100% xenon through a needle valve. Ether gas, 10%, (supplemented with oxygen) was also used as a positive control. The gas phase was replenished with fresh gas for 1 min every 1 h, and the container was mixed for 1 min. This entire procedure was repeated three times in order to saturate the liquid phase with respective gas at 25°C.

Since the low solubility of xenon in water-based solution might affect the results, polyethylene–glycol (PEG) was added to the reaction solutions, and the effect of xenon was reexamined under the same experimental conditions.

The hydrolysis by cholinesterase was started by adding 20 μl buffer solution containing 15 kBq *N*-[^{14}C]methylpiperidin-4-yl acetate or (R)-*N*-[^{14}C]methylpiperidin-3-yl butyrate (specific activity, 2.04 GBq/mM). The final concentration of *N*-[^{14}C]methylpiperidin-4-yl acetate or (R)-*N*-[^{14}C]methylpiperidin-3-yl butyrate was 0.033 mM in the reaction tube. The reaction was terminated by adding 400 μl ethanol after appropriate time intervals (30–1,800 s). These time intervals were sufficient to ensure that at least 50% of the radiotracer reacted in the respective solutions. The solution was centrifuged, and the supernatant was applied to a silica gel, thin-layer chromatographic plate and developed with a mixture of ethyl acetate, isopropanol, and ammonia (15:5:1). The thin-layer chromatographic plate was contacted with an imaging phosphor plate. The radioactivity corresponding to the unchanged ester and the hydrolyzed alcohol developed on the imaging phosphor plate was visualized and quantified using a bioimaging analyzer (BAS 2000 system; Fuji Photo Film, Tokyo, Japan).

When the substrate concentration ([S]) is much lower than the Km value in the Michaelis-Menten equation of enzymatic reaction, the reaction kinetics follows first order with a rate constant (Vmax/Km); $v = -d[S]/dt = Vmax/Km[S]$.

The substrate concentration was 0.033 mM, which is considered to be sufficiently smaller than the Km for the reaction to follow first-order kinetics, since the Km value of *N*-[^{14}C]methylpiperidin-4-yl acetate is 2.35 mM, for instance. It was also experimentally confirmed that both tracers disappear in a first-order process under the reaction condition in the range of the existent fraction from 0.9 to 0.1.

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Table 1. Cholinesterases Hydrolysis Constant and Inhibition for All Tissue solutions

	Brain		Blood		Brain PEG
	MP4A	MP3B_R	MP4A	MP3B_R	MP4A
Control	—	—	—	—	—
K	1.41 ± 0.03	0.21 ± 0.01	0.30 ± 0.02	0.35 ± 0.01	1.60 ± 0.15
Ether	—	—	—	—	—
K	0.23 ± 0.01*	0.08 ± 0.02*	0.09 ± 0.03*	0.07 ± 0.01*	
Inhibition, %	83	63	70	80	
Xenon	—	—	—	—	—
K	1.51 ± 0.09	0.23 ± 0.01	0.32 ± 0.03	0.32 ± 0.04	1.61 ± 0.10
Inhibition, %	−7	−8	−6	9	−0.6

Data shown as mean ± SD. Inhibitory effect of xenon on cholinesterases was compared with ether in brain tissue homogenate solution and blood. PEG was added in brain tissue homogenate solution to ensure the effect of xenon by increasing solubility of xenon.

MP4A and MP3B_R are radiotracer analogs of acetylcholine (*N*-[^{14}C]methylpiperidin-4-yl acetate) and butylcholine ((R)-*N*-[^{14}C]methylpiperidin-3-yl butyrate), respectively.

* $P < 0.0001$ (control, xenon *vs*. ether).

K, cholinesterases hydrolysis constant ($min^{-1} \cdot g^{-1} \cdot ml^{-1}$); PEG, polyethylene glycol.

The enzymatic hydrolysis rate constant (Vmax/Km) of each tracer was calculated and normalized by the tissue concentration as *K* ($min^{-1} \cdot g^{-1} \cdot ml^{-1}$) as follows:

$$K = -\ln(A_2/A_1)/(T_2 - T_1)/C$$

where A_1 and A_2 represent the ^{14}C radioactivity fraction of the unchanged ester remaining at times T_1 and T_2, respectively. T_1 and T_2 are the two time points at which the hydrolysis reactions were terminated in each solution within the range mentioned previously, and C represents the brain tissue concentration (in grams per milliliter) in the reaction solution. Inhibition was calculated as follows:

$$\text{Inhibition (\%)} = (1 - K/K_{\text{control}}) \times 100$$

Data were described as mean ± SD from duplicate samples from each of three animals in experiments without PEG or from five animals in the experiment with PEG added. One-way analysis of variance was used, followed by Scheffé *post hoc* test for pairwise comparison. A *P* value less than 0.05 was considered statistically significant.

Results

As expected, ether inhibited both acetylcholinesterase and butyrylcholinesterase in all groups (brain and blood) compared with control (table 1). Inhibition was 83% for brain acetylcholinesterase, 63% for brain butyrylcholinesterase, 70% for blood acetylcholinesterase, and 80% for blood butyrylcholinesterase. The differences in the hydrolysis constant between the control and ether groups were all statistically significant ($P < 0.0001$).

Conversely, inhibition by xenon was not significantly different from the control group for all tissue solutions (table 1). The addition of PEG to the brain tissue solution had no effect on cholinesterase inhibition by xenon (table 1).

These data show that xenon at 1 atm has no significant effect on cholinesterases in rat brain tissue and blood *in vitro*.

Discussion

Although we postulated that the cholinergic effects of xenon anesthesia might be due to the inhibition of cholinesterases, as shown with ether and cyclopropane in the 1970s,[4,5] our data demonstrate that xenon has no significant effect on cholinesterases *in vitro*. Thus, we believe that the cholinergic effects or increased acetylcholine concentration in the brain resulting from the administration of xenon are due to an increased release of acetylcholine from the cholinergic nervous system. In conclusion, xenon anesthesia does not affect cholinesterases *in vitro*.

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