Xenon inhalation increases norepinephrine release from the anterior and posterior hypothalamus in rats

[L'inhalation de xénon augmente la libération de noradrénaline de l'hypothalamus antérieur et postérieur chez les rats]

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Purpose: To investigate the effect of xenon (Xe) and nitrous oxide (N_2O) on norepinephrinergic neuronal activity in the rat medial preoptic area (mPOA) and posterior hypothalamus (PH) using microdialysis.

Methods: Sixty male Wistar rats were equally allocated to two groups: mPOA and PH. A microdialysis probe was implanted into the mPOA or the PH. In both groups, each animal was exposed to one of the following inhalations: 25% oxygen (control, $n=6$), 30% Xe ($n=6$), 60% Xe ($n=6$), 30% N_2O ($n=6$) or 60% N_2O ($n=6$). Norepinephrine concentration in the perfused artificial cerebrospinal fluid was measured by high pressure liquid chromatography at ten-minute intervals. After plotting the time-norepinephrine concentration curve, the area under the curve (AUC) in each group was calculated.

Results: In the mPOA, 30 and 60% Xe, but only 60% N_2O significantly increased norepinephrine release. The AUC in the 30% Xe, 60% Xe or 60% N_2O group was 160 ± 9 ($P<0.05$), 288 ± 42 ($P<0.01$) or 237 ± 46 pg·min/sample ($P<0.01$), respectively, compared to that in the control group: 77 ± 14 pg·min/sample. In the PH, only 60% Xe significantly increased norepinephrine release compared to control (AUC: 191 ± 38 *vs* 71 ± 1 pg·min/sample, $P<0.01$).

Conclusion: The present data suggest that Xe stimulates norepinephrinergic neurons more potently than N_2O; 1.2 times more in the mPOA and 2.5 times more in the PH. This stimulant effect may contribute to the hypnotic and sympathotonic effects of Xe in rats.

Objectif : *Rechercher, à l'aide de la microdialyse, l'effet du xénon (Xe) et du protoxyde d'azote (N2O) sur l'activité neuronale de la noradrénaline dans l'aire préoptique médiane (APOm) et dans l'hypothalamus postérieur (HP) de rats.*

Méthode : *Soixante rats mâles Wistar ont été répartis également en deux groupes: APOm et HP. Une sonde à microdialyse a été implantée dans l'APOm et l'HP. Chaque animal a été exposé à l'une des inhalations suivantes : 25 % d'oxygène (témoin, n = 6), 30 % de Xe (n = 6), 60 % de Xe (n = 6), 30 % de N2O (n = 6) ou 60 % de N2O (n = 6). La concentration de noradrénaline du liquide céphalo-rachidien artificiel perfusé a été mesurée par chromatographie liquide haute performance à des intervalles de dix minutes. Après avoir tracé la courbe de la concentration de noradrénaline en fonction du temps, on a calculé l'aire sous la courbe (ASC) pour chaque groupe.*

Résultats : *Dans l'APOm, les concentrations de 30 et 60 % de Xe, et de 60 % seulement de N_2O, ont augmenté significativement la libération de noradrénaline. Les ASC dans les groupes à 30 % de Xe, 60 % de Xe ou 60 % de N2O a été de 160 ± 9 (P<0,05), 288 ± 42 (P <0,01) ou 237 ± 46 pg·min/échantillon (P <0,01), respectivement, comparés à celle du groupe témoin : 77 ± 14 pg·min/échantillon. Dans l'HP, seul le Xe à 60 % a augmenté sensiblement la noradrénaline comparé au groupe témoin (ASC : 191 ± 38 vs 71 ± 1 pg·min/échantillon, P <0,01).*

Conclusion : *Les présentes données suggèrent que le Xe stimule les neurones noradrénalinergiques de façon plus importante que le N_2O, soit 1,2 fois plus dans l'APOm et 2,5 fois plus dans l'HP. Cet effet stimulant peut contribuer aux effets hypnotique et sympathicotonique du Xe chez les rats.*

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AN ideal inhaled anesthetic agent requires the following properties:[1] it should be stable, non-explosive and non- toxic; it should possess a low blood/gas partition coefficient, minimal cardiovascular and respiratory effects. Although nitrous oxide (N_2O) has minimal cardiovascular and respiratory effects, its anesthetic potency is not sufficient to provide adequate anesthetic depth by itself. Xenon (Xe) is a more potent anesthetic with no occupational and environmental disadvantages.[2] Therefore, Xe could be an alternative to N_2O although it is expensive.

The neurotransmitter, norepinephrine, is thought to play an important role in the regulation of physiological functions such as consciousness and autonomic nervous control.[3,4] A previous report[5] suggested that duration of anesthesia induced by barbiturates, chloral hydrate and propofol may be related to norepinephrinergic neuronal activity. Using a microdialysis method, we have studied the effects of several general anesthetic agents such as sevoflurane,[6,7] halothane,[7] ketamine,[8,9] midazolam[9] and propofol[9] on norepinephrine release from the posterior hypothalamus (PH) or medial prefrontal cortex.

The hypothalamus is a crucial homeostatic centre in the brain and its norepinephrinergic neuronal activity is closely related to physiological variables, including the regulation of consciousness and hemodynamics. The medial preoptic area (mPOA) in the anterior hypothalamus is thought to regulate consciousness, since reports using electrophysiological[10] and micro injection technique[3,11] suggest that norepinephrinergic neuronal activity in the mPOA modulates sleep-wakefulness. In addition, Osaka *et al.*[10] clearly showed that sleep-related neurons exist in the mPOA. The PH is involved in the regulation of the autonomic nervous system.[12] An elevation in norepinephrine concentration in the PH increases sympathetic tone.[12,13]

General anesthetic agents are known to modulate consciousness and hemodynamics. As described above, it is likely that norepinephrinergic neuronal activities in the hypothalamus play an important role in the modulation of consciousness and hemodynamics. This is why we investigated the effects of Xe on norepinephrine release from the mPOA and PH using a microdialysis technique, and compared them with those of N_2O.

Methods

The study was approved by the animal experiment committee of our institution. Sixty male Wistar rats weighing 270–330 g were randomly assigned to two groups: mPOA (n=30) and PH (n=30). They were housed for at least a week before the implantation surgery. They were kept in a 12-hr light-dark cycle environment (lights on at 0800 AM) at a temperature of 23 ± 1°C. They had free access to food and water except on the day of the experiment.

A microdialysis probe with a 2-mm long semipermeable membrane in its tip (A-I-12-2, EICOM, Kyoto, Japan) was implanted via a guide cannula (AG-12, EICOM, Kyoto, Japan) following pentobarbitone anesthesia (50 $mg \cdot kg^{-1}$ *ip*) into the mPOA with the following coordinates (A: -0.92, L: 2.5 at an angle 11 from the bregma, V: 8.5 mm from the brain surface) or into the PH (A: -3.6, L: 1.3, V: 9.5 mm from the bregma) according to the atlas by Paxinos.[14]

Animals were allowed to recover for 24 hr following probe implantation. The probe was perfused at a rate of 1.3 $\mu L \cdot min^{-1}$ with artificial cerebrospinal fluid (NaCl 128 mM; KCl 2.6 mM; $CaCl_2$ 1.3 mM; $MgCl_2$ 0.9 mM; $NaHCO_3$ 20 mM; Na_2HPO_4 1.3 mM) containing 1 mM pargyline to prevent degradation of norepinephrine. Each animal was placed in a custom-built Plexiglas box in which the animal could move freely. Oxygen concentration in the box was maintained at 25% throughout the experiment to prevent hypoxia. After an equilibration period, the dialysates were collected at ten-minute intervals. After obtaining five consecutive samples, each animal was exposed to one of the following gas mixtures for 30 min: 25% oxygen (control, n=6), 30% (n=6) or 60% (n=6) Xe (Nippon Sanso Co., Tokyo, Japan), or 30% (n=6) or 60% (n=6) N_2O. After the end of each inhalation, five more samples were obtained. On-line gas monitors (Xenon Gas Monitor™, ANZAI, Tokyo, Japan; Capnomac™, IMI, Tokyo, Japan) continuously monitored the concentrations of Xe, N_2O and oxygen in the box.

All animals exposed to 60% Xe (n=12) or 60% N_2O (n=12) were observed for loss of righting reflexes. The loss of righting reflex was defined as loss of ability to perform three successive rightings.

The norepinephrine concentration was measured by a high-performance liquid chromatography with an electrochemical detector as described previously.[8,9] Briefly, the 10 µL samples were injected into ODS-C18 reverse- phase column (CA-5ODS, EICOM, Kyoto, Japan) maintained at 25C. The mobile phase was 0.1 M phosphate buffer containing 5% methanol and its flow was 220 $\mu L \cdot min^{-1}$. The oxidation potential of the graphite electrode was set at +400 mV against an Ag/AgCl reference electrode. The detection limit of the assay was 125 fg·10 µL (signal/noise ratio >3).

All data were expressed as mean ± SEM. The area under the curve (AUC) of the norepinephrine concentration from 0 to 80 min during and after drug

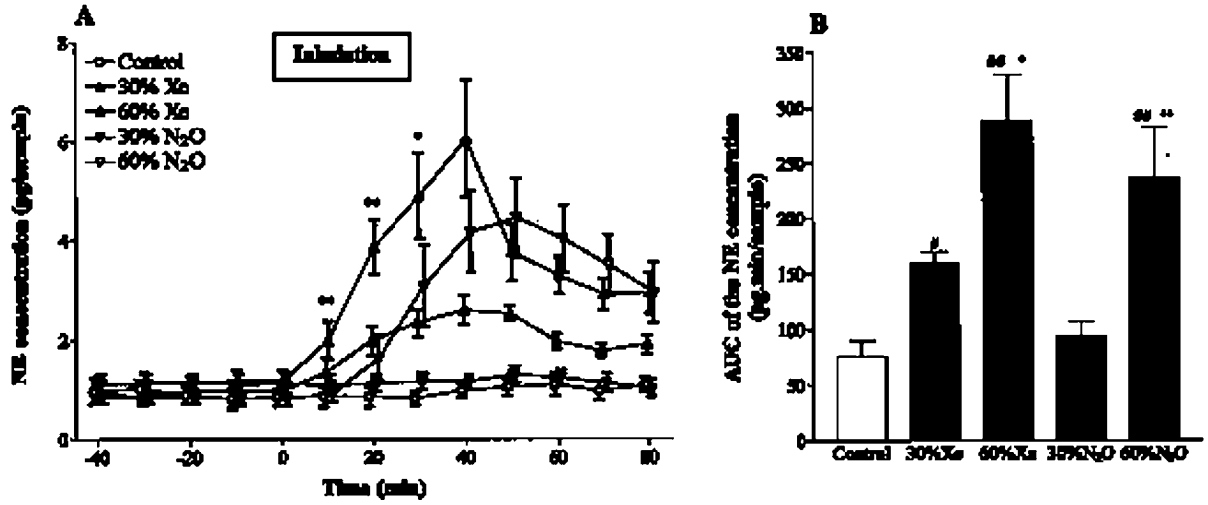

FIGURE 1 Changes in norepinephrine (NE) release from the medial preoptic area during and after xenon (Xe) or nitrous oxide (N_2O). A, Time course data. Data are expressed as mean ± SEM. Control (25% oxygen; , n=6), 30% Xe (, n=6), 60% Xe (Δ, n=6), 30% N_2O(, n=6), 60% N_2O(▽, n=6). Rats were exposed to each drug from time 0 to 30 min. *P <0.05, **P <0.01 *vs* 60% N_2O group. At these points, NE concentrations are also different from those in control (P<0.05). B, Area under the curve (AUC) of NE release from the medial preoptic area measured from 0 to 80 min after start of inhalation of drugs. Data are expressed as mean ± SEM. Control (25% oxygen; n=6), 30% Xe (n=6), 60% Xe (n=6), 30% N_2O (n=6), 60% N_2O (n=6). #P <0.05, ##P <0.01 *vs* control in the same region; ^{+}P <0.05, ^{++}P <0.01 *vs* the AUC for the drugs in the posterior hypothalamus.

exposure was measured with computer software (GraphPad Prism 1.0). One-way ANOVA followed by Fisher's PLSD was used for appropriate inter-group comparisons. Fisher's exact probability test was used to compare the proportion of animals with loss of righting reflex in the 60% Xe or 60% N_2O group. AP <0.05 was considered significant.

Results

In the control group (25% oxygen), the basal norepinephrine release (-40 to 0 min) from the mPOA and PH were 0.9 ± 0.1 and 0.8 ± 0.1 pg/sample, respectively (Figures 1A, 2A). There were no significant differences in the basal norepinephrine release among groups and between two brain regions. However Xe or N_2O-evoked norepinephrine release in the mPOA was significantly greater than that in the PH. (Figures 1B, 2B).

In the mPOA, the AUC of norepinephrine concentration from 0 to 80 min after the start of inhalation showed that norepinephrine release following inhalation of 30 and 60% Xe and 60% N_2O significantly increased compared to that in the control group (control, 76 ± 16; 30% Xe, 158 ± 9; 60% Xe, 282 ± 41 pg·min/sample, Figure 1B). In addition, norepinephrine release during inhalation of 60% Xe was significantly greater than during inhalation of 60% N_2O (Figure 1A). An increase in norepinephrine release from the PH was observed only in the 60% Xe group (Figure 2AB). This increase was significant, compared to the 60% N_2O group (60% Xe, 192 ± 37; 60% N_2O, 73 ± 9 pg·min/sample, Figure 2AB).

With regard to changes in behaviour, righting reflex was lost in six of 12 rats in the 60% Xe group. Another six rats appeared well sedated as they rarely moved, but their righting reflex was still preserved. In contrast, no rats in the 60% N_2O group lost its righting reflex, and appeared sedated. Change in behaviour was significantly different between groups (Table, P <0.01).

Discussion

We report the effect of Xe on norepinephrinergic neuronal activity in the hypothalamus. We observed that Xe significantly increased norepinephrine release in the mPOA and PH, while N_2O did so only in the mPOA. The diversity may be based on their anesthetic potency. Minimal alveolar concentration (MAC) of Xe is 71% and that of N_2O is 105% in humans.[2] In addition, the increase in norepinephrine release in the mPOA was more pronounced than in the PH. The mPOA is thought to be involved in the neuronal circuit eliciting the regulation of consciousness.[3,10] Previous investiga-

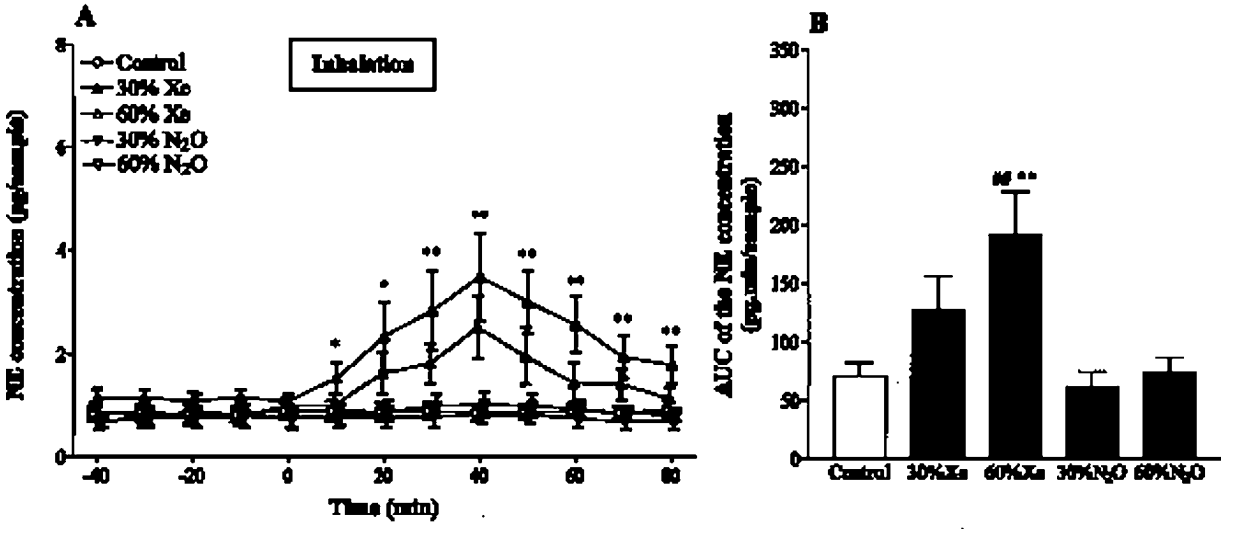

FIGURE 2 Changes in norepinephrine (NE) release from the posterior hypothalamus during and after xenon (Xe) or nitrous oxide (N_2O). A, Time course data. Data are expressed as mean ± SEM. Control (25% oxygen; , n=6), 30% Xe (, n=6), 60% Xe (Δ, n=6), 30% N_2O (, n=6), 60% N_2O (∇, n=6). Rats were exposed to each drug from time 0 to 30 min. *P<0.05, **P<0.01 *vs* 60% N_2O. At these points, NE concentrations are also significantly different from those in control (P<0.05). B, Area under the curve (AUC) of the NE release from the posterior hypothalamus measured from 0 to 80 min after start of inhalation of drugs. Data are expressed as mean ± SEM. Control (25% oxygen; n=6), 30% Xe (n=6), 60% Xe (n=6), 30% N_2O (n=6), 60% N_2O (n=6). ##P<0.01 *vs* control; **P<0.01 *vs* 60% N_2O in the same region.

TABLE Effects of 60% Xe and N_2O on righting reflex

Group	*Righting reflex*	
	(+)	*(-)*
60% Xe (n=12)	6	6
60% N_2O (n=12)	12	0

Xe=Xenon; N_2O=nitrous oxide; (+)=preservation of the reflex, (-)=loss of the reflex, P<0.01 between groups.

tors[3] have shown that increases and decreases in norepinephrine concentration in the mPOA cause arousal and sedation, respectively. Interestingly, there are reports suggesting that an increase in some norepinephrinergic neuronal activity in the mPOA may induce sleep. Hagemann and colleagues[16] reported that norepinephrine microinjection into the area could increase the duration of sleep in pigeons. Kumar *et al.*[11] also demonstrated the sleep-inducing function of norepinephrinergic fibres in the mPOA. In their report, when the presynaptic norepinephrine terminals in the mPOA were destroyed, the microinjection of norepinephrine into the mPOA induced sleep. These findings indicate that some norepinephrinergic neurons in the mPOA may be involved in hypnogenesis. Mizuno and colleagues[15] reported that norepinephrine release in the mPOA decreased following pentobarbitone *ip*. In contrast, we observed that both Xe and N_2O significantly increased norepinephrine release. Similarly, we reported previously that an anesthetic dose of ketamine also significantly increased norepinephrine release in the medial prefrontal cortex while an anesthetic dose of midazolam and propofol decreased the release of norepinephrine.[9] Therefore, some general anesthetics may increase norepinephrine release with anesthesia, while others will decrease it.

We observed that all animals were well sedated during the inhalation of 60% Xe, but not during inhalation of 60% N_2O. In addition, in the mPOA, the increase in norepinephrine release during inhalation of 60% Xe was significantly greater than that during 60% N_2O. Our results suggest that animals become sedated when norepinephrine release exceeds a specific concentration. Further studies will be required to elucidate the exact relation between Xe anesthesia and norepinephrinergic neurons in the mPOA.

Xe significantly increased norepinephrine release in the PH, a region known to regulate sympathetic nervous system activity.[12] In addition, norepinephrine concentration in this region parallels sympathomimetic

tone.[12,13] Thus, our data suggest that Xe may stimulate sympathetic nervous system activity. Similarly, Webster and colleagues[17] reported that Xe increased arterial pressure in rats. However, as clinical reports in humans suggest that Xe may not cause sympathetic activation,[2] the effects of Xe on sympathetic nervous activity may be different in rats and in humans. In contrast, although N_2O is reported to stimulate the sympathetic nervous system,[18] it did not increase norepinephrine release in the PH. The MAC of N_2O in rats has been reported to be 221%.[19] In the present study, as rats inhaled only 60% N_2O, this concentration may not have been sufficient to increase norepinephrine release in the PH.

In conclusion, the present study suggests that Xe increases norepinephrine release in the hypothalamus more potently than N_2O does. This increase may contribute to the anesthetic effects of Xe.

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