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The relationship of brain catecholamine levels to enflurane requirements among three strains of mice with different anesthetic sensitivities

Hiroyuki Nakao[1], Junichiro Ono[1], Junko Nogaya[1], Satoshi Yokono[2], and Kouichi Yube[3]

[1] Department of Anesthesiology and Emergency Medicine, Kagawa Medical University, 1750-1 Ikenobe, Miki, Kita, Kagawa, 761-0793, Japan
[2] Department of Anesthesiology, Kyoto Second Red Cross Hospital, Japan
[3] Research Equipment Center of Kagawa Medical University, Japan

Abstract

Purpose. It has been reported that brain catecholamines alter the minimum alveolar concentration (MAC) of anesthetics. The extent of the relation between the levels of brain catecholamine and anesthetic sensitivity should be evaluated by excluding several factors.

Methods. Anesthetic sensitivity was measured by using loss of the righting reflex in three strains of mice with different sensitivities. The mice were decapitated without any anesthesia, adding on ddN and C57BL/6J mice in 2% enflurane, their brains were divided into three parts, and dopamine and norepinephrine levels were analyzed by high-performance liquid chromatography (HPLC).

Results. The values of enflurane requirement (%) were 1.30 ± 0.05 in ddN, 1.10 ± 0.02 in C57BL/6J, and 1.05 ± 0.02 in MSM mice. The values of dopamine ($\mu g \cdot g^{-1}$) in the mesencephalon were 0.23 ± 0.02 in ddN, 0.15 ± 0.02 in C57BL/6J, and 0.12 ± 0.02 in MSM (mean ± SE). No statistical significance in the values in 2% enflurane could be obtained between ddN and C57BL/6J. The stepwise regression line showed a significant correlation: enflurane requirement (%) $= -0.89 + 1.60 \times$ (dopamine levels of mesencephalon) ($r^2 = 0.571$, $P < 0.0001$).

Conclusion. Dopamine in the mesencephalon seems to play an important role in the production of different anesthetic sensitivities, and the anesthetic mechanism might be related to the regulation of dopamine levels that promote arousal.

Key words Anesthetics · Volatile · Enflurane · Brain · Mesencephalon · Dopamine · Potency · Enflurane requirement

Introduction

Loss of righting reflex (LORR) is one of the indices that shows the effective concentration of the anesthetic requirement in the mouse. The center of this reflex is the mesencephalon. This part includes the midbrain reticular formation activating system connected with consciousness. Tanaka et al. have reported that there are 20 to 30 cooperative factors in anesthesia, based on the anesthetic dose—LORR relation, including catecholamines [1].

Many researchers have attempted to clarify the relationship between anesthetic requirement with LORR and catecholamine levels in the brain by using an animal administered an agonist or antagonist for catecholamine receptors during anesthesia, or by comparing catecholamine levels before and during anesthesia in one strain of mice [2,3]. These reports have not always provided accurate physiological information, because of the side effects that these drugs have produced following administration to the whole body, or because of possible alteration of levels of other transmitters in the brain.

We should research the relationship between LORR and catecholamine levels in the nonanesthetized state so as not to produce secondary effects, and search for the parts of the brain and catecholamines in the brain participating in anesthesia. Accordingly, we examined the degree of contribution of catecholamine by comparing the anesthesia sensitivity with levels of catecholamine in the brain under nonanesthetized conditions in three strains of mice, each with a different sensitivity to the anesthetic.

Methods

This research was conducted with the approval of the Kagawa Medical University Ethics Committee. Three

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strains of male mice, ddN, C57BL/6J, and MSM, were used. Tanaka et al. reported that the ddN mouse was more resistant to three volatile anesthetics than the C57BL/6J mouse [1]. We decided to measure the enflurane requirement, because in enflurane, especially, the effective dose 50% (ED_{50}) values differed about 40% in this report, and because enflurane is more effective in the order of anesthetic sensitivity. The ddN mouse is considered an inbred strain, because these closed colony mice have been inbred for more than 20 generations. The 19 marker genes of the six ddN mice selected at random were equal, as determined by Dr. Hideki Kato of the Central Institute for Experimental Animals, Mishima, Japan. The C57BL/6J mice were purchased from Charles River (Wilmington, USA). The MSM mice were propagated naturally at the National Institute of Genetics. The body weights of the ddN and C57BL/6J laboratory mice were 25–30 g, and those of the MSM wild-type mouse were 10–15 g. The mice were 8–12 weeks old. They were bred under the same conditions of food availability, circadian rhythm, body temperature, humidity, brood, and sexual segregation, with 4–7 per cage. We removed an aggressive mouse (the alpha mouse) and an injured mouse from the experiment because stress may have affected their anesthetic sensitivities or brain catecholamines.

The enflurane sensitivities of 12 ddN mice, 17 C57BL/6J mice, and 14 MSM mice were examined over 3 h from 8 a.m. to 11 a.m. The anesthetic requirement was measured by using LORR. Each mouse was placed in an airtight plastic 25-l capacity box with an outlet and inlet for the anesthetic gas. Carbon dioxide was removed by a carbon dioxide adsorbent (Wako, Tokyo, Japan) in the box. The gas in the box was stirred with a fan to equalize the concentration. Anesthetic gas was adjusted by running air at $4\,l \cdot min^{-1}$ through the enflurane vaporizer. The concentration was checked with an anesthetic agent analog monitor (Datex, Normac, Finland). To maintain the rectal temperature, the plastic container was equipped with an electric heater to control the temperature within 28–30°C. Manipulations of the mice were conducted by gloved hands from outside the airtight container. An initial 30-min period of equilibration at an approximate level of 0.8% enflurane was imposed before testing the righting reflex. We regarded the mice as being in anesthetic equilibrium after 15 min of enflurane at a constant concentration [1]. LORR was determined to have occurred if the mouse did not voluntarily right itself when placed gently by hand in the dorsal position without any violent movement or painful stimulation. The enflurane requirement (ER) was determined as the concentration at which the mouse remained in the dorsal position for 10 s. The concentration of the anesthetic was gradually increased by 0.1% until LORR was confirmed.

Levels of catecholamine in the brain were measured 7 days after enflurane anesthesia in order to exclude any anesthetic effect. We removed four ddN mice, seven C57BL/6J mice, and five MSM mice because of aggression, injury, or death. Eight mice of the ddN group, 10 of the C57BL/6J group, and 9 of the MSM group were quickly decapitated with a large pair of scissors at room temperature between 11 a.m. and 3 p.m. The head was immediately carried to a 4°C room, and within 90 s the brain was removed from the skull and divided into the cerebrum, mesencephalon, and diencephalon. These three parts were immediately weighed and put into a cooling solution to dilute 200 times to produce an appropriate catecholamine measuring range [4]. The cooling solution for the brain parts of the decapitated mice consisted of 60% perchloric acid 34 ml, ethylene diamine tetraacetic acid disodium salt (EDTA2Na) 0.38 g, L-ascorbic acid 0.19 g, and distilled water 1 l. The cooling solution was based on the method of Refshange and Kissinger [4,5] as a pretreatment for preventing metabolism and degeneration of the catecholamine. Each part was homogenized at 1300 rotations per minute, four strokes, 4°C within 3 min from decapitation, and centrifuged at 5000 *g* while being cooled for 30 min. Brain catecholamines begin metabolism 5 min after death [6]. The supernatant was then analyzed with a Tosho model HLC-725CA (Tokyo, Japan) fully automated catecholamine analyzer [5,7]. The levels of catecholamine were shown as mean ±SE ($\mu g \cdot g^{-1}$ wet brain). We also examined the three parts of other brains under the microscope to verify that the parts were accurately divided so as not to affect the levels of catecholamine.

Two strains of mice with different anesthetic sensitivities, ddN and C57BL/6J, were decapitated after being subjected to 2% enflurane for 30 min as a pilot study, and the catecholamine levels in their brains were measured by the same method. This concentration was enough to anesthetize the two strains of mice.

The statistical analysis was conducted with StatView software version 4.5 (Abacus Concepts, Berkeley, CA, USA) using analysis of variance (ANOVA) and Scheffe's *F* test as a post hoc test. The data are displayed as mean value ± standard error (sample number), and $P < 0.05$ was considered significant. Stepwise regression analysis was also conducted for limiting independent variables with forward selection procedure and backward elimination procedures. This regression was analyzed to determine the association with ER among noradrenaline (NA) and dopamine (DA) in the cerebrum, NA and DA in the mesencephalon, and NA and DA in the diencephalon. The significance level was set at $P < 0.05$, the accepted *F* value was 4.000, and the excluded *F* value was 3.996.

Results

The ER (%) values were 1.30 ± 0.05 in ddN, 1.10 ± 0.02 in C57BL/6J, and 1.05 ± 0.02 in MSM mice. Table 1 shows a summary of the statistics of the variables analyzed. There were significant differences in ER between ddN and the other two strains of mice ($P < 0.05$).

The experimental results are shown in Fig. 1. There were significant differences in catecholamines in the parts of the brain among ddN, C57BL/6J, and MSM mice ($P < 0.05$). In the three strains of male mice, the levels of catecholamine decreased with the decrease in anesthesia requirement.

Table 1. Enflurane requirement (ER) of three mouse strains

Strain	ER
ddN	1.3 ± 0.05(12)
C57BL/6J	1.10 ± 0.02(17)*
MSM	1.05 ± 0.02(14)*,**

Mean ± SE (%) (N, number of mice)
*Significant difference as compared with ddN ($P < 0.05$); **significant difference as compared with C57BL/6J ($P < 0.05$)

No statistically significant differences in the levels of catecholamine in 2% enflurane could be obtained between ddN and C57BL/6J in any brain region: cerebrum (NE: ddN 0.22 ± 0.01(5), C57BL/6J 0.24 ± 0.01(5)), (DA: ddN 0.97 ± 0.04 (5), C57BL/6J 1.02 ± 0.04(5)), diencephalon NE: ddN 0.54 ± 0.06(5), C57BL/6J 0.65 ± 0.05(5), (DA: ddN 0.18 ± 0.04(5), C57BL/6J 0.16 ± 0.02 (5)), or mesencephalon (NE: ddN 0.78 ± 0.04(5), C57BL/6J 0.89 ± 0.08(5)), (DA: ddN 0.45 ± 0.05 (5), C57BL/6J 0.36 ± 0.05 (5)) (mean ± SE ($\mu g \cdot g^{-1}$ wet brain) (the number of mice)).

The stepwise regression analysis of the relationship between ER and catecholamines advanced to five steps with both the forward selection procedure and the backward elimination procedure, and the only accepted variable was DA in the mesencephalon. The regression line derived from the plot of ER versus DA levels in the mesencephalon showed a highly significant correlation: ER (%) = −0.89 + 1.60 × (DA levels of mesencepha-

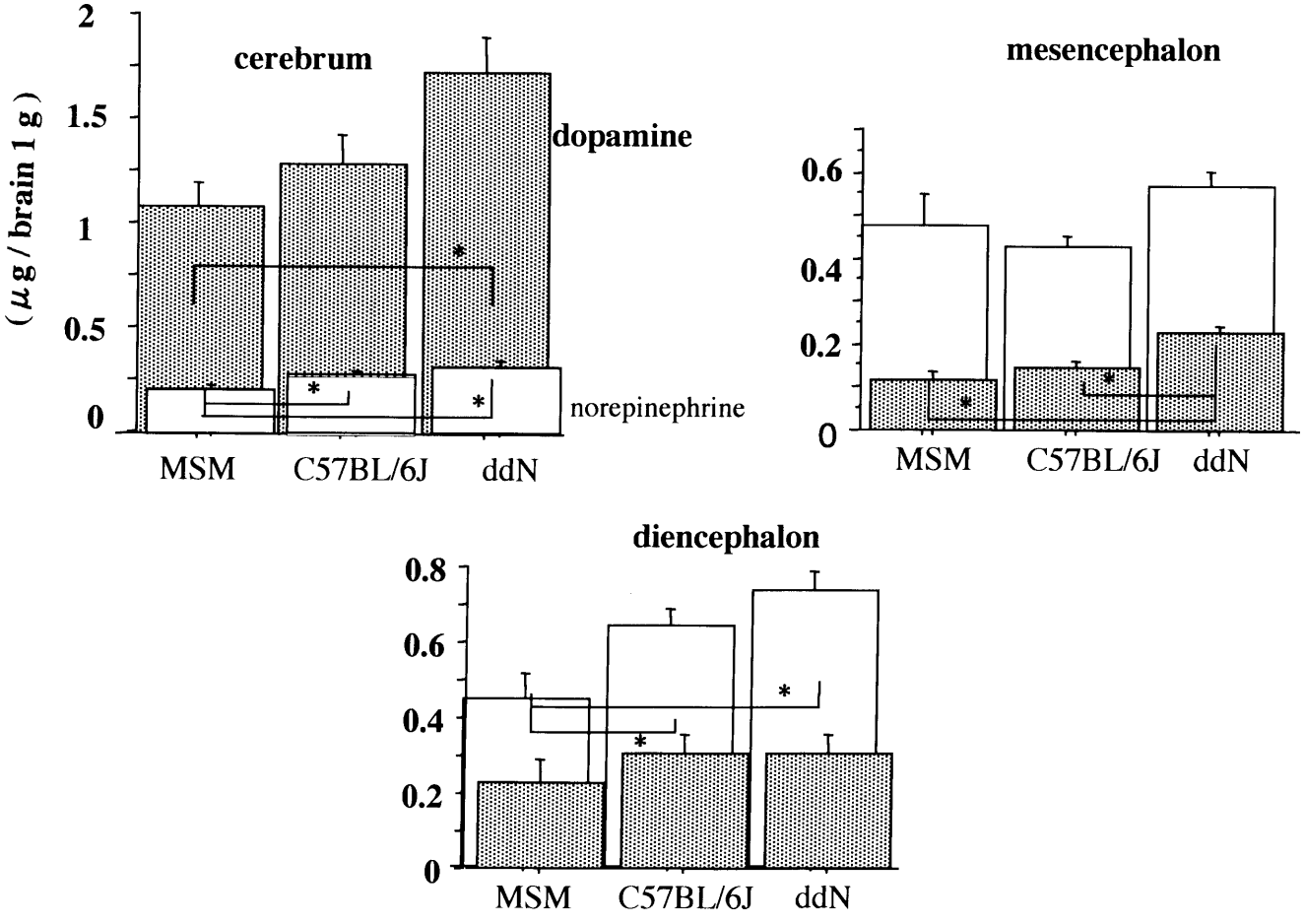

Fig. 1. Comparison of catecholamine levels in three mouse strains: ddN($n = 8$), C57BL/6J($n = 10$), and MSM($n = 9$). Values are means ± SE. *Scheffe's test: $P < 0.05$. *Shaded areas* indicate dopamine levels. *Unfilled areas* show norepinephrine levels

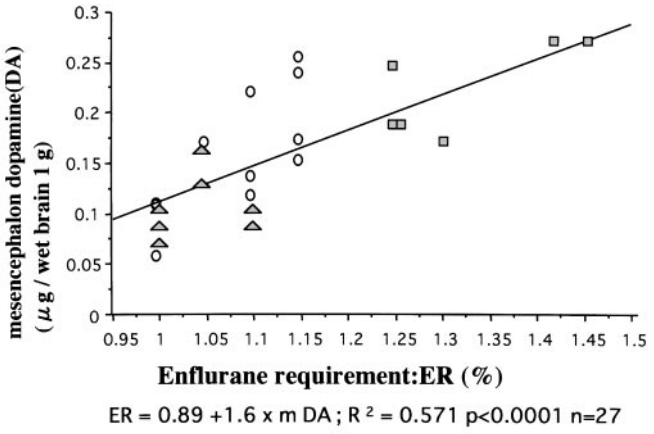

Fig. 2. Relationship of ER and DA in mesencephalon in three strains of mice: *squares*, ddN; *circles*, C57BL/6J; *triangles*, MSM

lon) ($r^2 = 0.571$, $P < 0.0001$) (Fig. 2). The correlation between enflurane requirement and catecholamine level in the three strains of male mice was significant for DA in the mesencephalon.

Discussion

Several neurotransmitters are connected with consciousness, because nerve fibers with neurotransmitters are found in arousal neural mechanisms and sleep neural mechanisms. These arousal neural mechanisms with their center in the brainstem are regulated by the aminergic and cholinergic modulating systems. It is said that aminergic neurons in this system include mainly serotonin, NA, and histamine. DA is metabolized to NA. DA may not be directly connected with the arousal neural mechanism in sleeping, but may indirectly affect the mechanism in anesthesia. Actually, it has been reported that the minimum alveolar concentration (MAC) is decreased by the dopamine antagonist, whereas MAC is increased by the dopamine agonist [2,3]. Also, Roizen reported that the anesthesia requirement decreased with destruction of the brainstem [8,9]. Also, substantia nigra, including dopaminergic neurons, connects fibers with the reticular formation.

It has been reported from electroencephalographic analysis that one is awake if the mesencephalon is electrically stimulated [10–13] and that one falls into a coma if this region is destroyed [8,10]. It seems that neurotransmitters are deeply involved in the mechanism of general anesthetic agents from the neurophysiological standpoint.

The idea of comparing levels of catecholamine in the brain with anesthetic sensitivity led, we believe, to the correct result. We found that no statistically significant differences in the levels of catecholamine in 2% enflurane could be obtained between ddN and C57BL/6J mice in any brain region. Other reports that have examined levels of catecholamine before and after anesthesia of only one strain of mouse have not necessarily used the correct methods to examine whether or not levels of catecholamine were related to anesthetic sensitivity [2,3]. For this reason, other reports could not check that the two strains of mice with different anesthetic sensitivities have the same levels in their brain catecholamines during anesthesia from our data. The levels of catecholamine in anesthesia from our data may be the result of the anesthetic and not due to the original levels of catecholamine in each mouse. Therefore, only a comparison of levels of catecholamine among strains of mice in the conscious state will largely reflect the relationship of the levels of catecholamine and anesthetic sensitivity. The correlation between ER and DA levels in the mesencephalon is significant, but there was no significant correlation in other parts of the brain. There were significant differences DA and NE levels in the cerebrum and NE in the diencephalon among the three strains of mice. We think that the other catecholamines except DA in the mesencephalon may be indirectly related to ER, because the order of ER in the three strains of mice was that of catecholamines in other parts of the brain. The other catecholamines may be affected by DA in the mesencephalon or by the other neurotransmitters.

There was a significant difference in DA levels in the mesencephalon between ddN mice and C57BL/6J mice without enflurane, but no statistically significant difference in the levels of catecholamine in 2% enflurane could be obtained between ddN and C57BL/6J in any brain region. The mice were very agitated at the lower concentration when enflurane was introduced, and such agitation can affect the levels of brain catecholamines [14]. We think that an increase of brain catecholamines from the agitation may mask the difference in brain catecholamines between the two strains of mice without enflurane. However, there are no reports on the effect of agitation on anesthetic requirement.

The levels of catecholamine during consciousness were measured in the cerebrum, mesencephalon, and diencephalon of the brains of the three strains of mice in which the anesthesia sensitivities differ. The number of mice in each strain differed. A sufficient number of C57BL/6J mice could be bred and purchased, but ddN and MSM mice did not breed as much as expected and could not be newly purchased. However we think that the numbers were adequate for statistical analysis, because there was no difference in their standard errors.

The level of catecholamine parallels the anesthetic requirement. The catecholamine measured in this paper

is the overall content, and not the specific intracellular or extracellular content of the nerve cell. That is to say, catecholamine levels were totaled from the quantity of synaptic vesicles in the nerve ending, the quantity secreted in the synaptic cleft, and the decomposition and uptake of catecholamine. The pellet sample was examined by electron microscope in order to verify that the synaptic vesicles were destroyed, so the vesicles could not be confirmed in the sample. But we do think it important to study levels of intracellular and extracellular catecholamine also.

The change of catecholamine levels in the brain before and after the use of anesthetic is not necessarily linked directly with the anesthetic requirement for LORR because of the effect on LORR of any change in locomotor activity and excitement during anesthetic induction. LORR may be insufficient as an index of anesthetic requirement, but that raises the question of what are the most suitable indexes of anesthetic requirement. For example, the tail clamp method involves pain. Some studies used the time of LORR as an index of anesthetic requirement. What does physiologically the time of LORR mean?

Koblin has also mentioned body temperature, blood pressure, age, ion concentration, and neurotransmitters as factors that influence the anesthetic requirement [15]. Therefore, it is important to examine the relationship between the depth of anesthesia and the levels of catecholamine in the brain. The locomotor activity of the mouse is minimized between 11 a.m. and 3 p.m., according to T. Koide of the National Institute of Genetics and K. Tsuji [16]. This time zone with the smallest locomotor activity was chosen so that we could rapidly decapitate the mice without inflicting too much pain.

DA levels of the mesencephalon have been found to be closely related to arousal by electrophysiological investigation and experimental evidence involving decerebration [17]. Although anesthetics are allosteric agonists of $GABA_A$ receptors, etc., we suspect that DA might modify anesthesia via the arousal mechanism, like the relationship between an accelerator and a brake. It has been pointed out that many neurotransmitters (acetylcholine, GABA, etc.) participate in anesthetic effects. However, we conclude that DA in the mesencephalon is an especially important factor in producing anesthesia. According to the relationship between DA and ER in the three strains of mice, the anesthetic mechanism is probably related to the regulation of DA levels that promote consciousness.

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