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Short communications

Effect of isolation stress on anesthetic requirement in mice

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Many studies have measured anesthetic requirements in order to determine anesthetic potency. The measurements in mice have been used to clarify anesthetic mechanisms, using the index for loss of righting reflex (LORR) [1] and the tail-flick test. However, many factors can affect anesthetic requirements [2]. Researchers have found widely divergent values for anesthetic requirements of mice. Male mice by nature suffer chronic stress because they tend to fight over territory in their cages, occasionally to the death [3]. There are no studies examining the effects of such stress on anesthetic sensitivity in mice. In particular, researchers have not paid attention to isolation stress when measuring anesthetic sensitivity.

We classified male mice into two groups, grouped mice (GM) and isolated mice (IM), and evaluated how isolation stress affected enflurane requirements.

This research was conducted with the approval of the Kagawa Medical University Ethics Committee. The ddN mice, MSM mice, and C57BL/6J mice were all male, 8–12 weeks old, and bred under the same conditions. IM were quarantined at 4 weeks after birth from the brothers (GM) and single parents to except genetic and environmental factors. The ddN mice were delivered less than the other strains. Because GM scuffle and injure each other when housed at less than three animals per cage, we decided to house them at more than four animals per cage (Table 1) [4].

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The enflurane sensitivities of the three strains of mice were examined with respect to LORR over a period of 3 h from 8 to 11 a.m. in a container [5]. The plastic container had a fan to equalize the anesthetic concentration and a carbon dioxide adsorbent, and the concentration was continuously monitored with an anesthetic agent monitor (Datex, Normac, Finland). Any manipulation of the mice was conducted with a glove from outside the airtight container, with the temperature kept between 28° and 30°C. We regarded the mice as being in anesthetic equilibrium after 15 min of enflurane at a constant concentration. LORR was determined to have occurred if the mouse did not voluntarily right itself when it was 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 enflurane was gradually increased by 0.1% until LORR was confirmed.

The results were processed with StatView, version 4.5 (Abacus Concepts, Berkeley, CA, USA) using analysis of variance (ANOVA) and the unpaired *t*-test as a post hoc test. The data are shown as means ± SD. $P < 0.001$ was considered to indicate statistical significance.

The ER values in GM and IM were 1.30% ± 0.07% and 1.69% ± 0.05%, respectively, in ddN mice; 1.02% ± 0.08% and 1.26% ± 0.05% in MSM mice; and 1.09% ± 0.05% and 1.32% ± 0.04% in C57BL/6J mice (Fig. 1). The ratio of ER values in IM to those in GM was 1.30 for ddN mice, 1.24 for MSM mice, and 1.21 for C57BL/6J mice.

The ER in IM was about 1.2–1.3 times higher than that in GM in ddN, MSM, and C57BL/6J mice. Norepinephrine levels are particularly related to threat and offense [6–8]. We suspected the following reasons for the differences between the ER values of IM and GM. We preliminarily studied brain norepinephrine levels in ddN mice and C57BL/6J mice by HPLC (high-

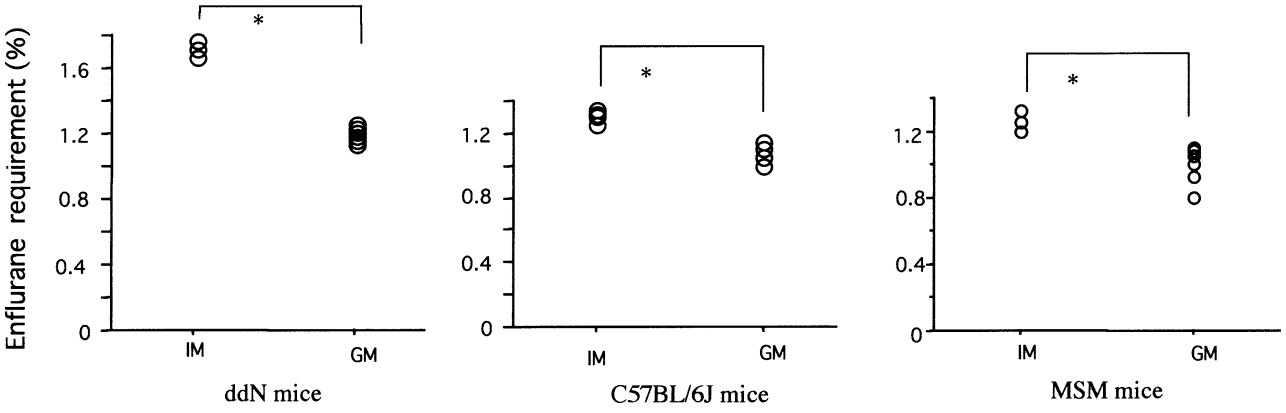

Fig. 1. Relationship between the enflurane requirement (ER) and stress conditions in the three strains of mice. *IM*, isolatied mice; *GM*, grouped mice without wounds. ○, ER of a mouse. In both strains, the ratio of ER values in *IM* to those in *GM* was about 1.21–1.30. The results were processed using analysis of variance (ANOVA) and the unpaired *t*-test as a post hoc test. The data are shown as means ± SD, and $P < 0.001$ was adopted as indicating a statistically significant difference

Table 1. Environmental conditions of isolated (IM) and grouped (GM) mice[a]

Strain	IM	GM
ddN	[1], [1], [1], [1]	[4], [4]
C57BL/6J	[1], [1], [1], [1], [1]	[4], [5], [6]
MSM	[1], [1], [1], [1], [1]	[4], [4], [5]

[a] [ ]: cage and number of mice in a cage

performance liquid chromatography) [9]. ddN: cNE (IM / GM 0.170 ± 0.027 / 0.278 ± 0.061), mNE (IM / GM 0.326 ± 0.157 / 0.425 ± 0.100), dNE (IM / GM 0.762 ± 0.227 / 0.641 ± 0.159); C57BL/6J: cNE (IM / GM 0.167 ± 0.019 / 0.320 ± 0.060), mNE (IM / GM 0.252 ± 0.071 / 0.570 ± 0.081), dNE (IM / GM 0.782 ± 0.165 / 0.733 ± 0.118), where c: cerebrum, d: diencephalon, m: mesencephalon, NE: norepinephrine, mean ± SD (μg per gram of wet brain). NE levels in the cerebrum were lower in IM than in GM in both ddN and C57BL/6J mice. Reis reported that threat increased the turnover of NE and reduced NE levels [7]. However, the correlation between NE and ER was not significant in either strain. Stress can also change catecholamine levels in the brain [10–12]. Anesthetic sensitivity can be affected by changing levels of brain catecholamines, caused by stress from population density. We suspect that ER under stress may be changed by neurotransmitters. For example, the concentration of serotonin is low in the brains of isolated mice [7]. Many neurotransmitters respond to stress.

NE in particular drives mice to perform threatening behavior or attack [13]. It has been reported that IM attack other mice more aggressively than GM [6].

Furthermore, mice in nature are timid and hide in bushes. GM are more resistant than IM to attack from invaders. Thus, IM are more cautious and more sensitive than GM. We suspect that the ratio we found might apply to other strains with respect to enflurane sensitivities. The stress of isolation on the weak in nature may be the same regardless of the strain of mouse. The dominant mice in an area, which cannot be attacked by other animals, probably have a different ratio of ER from GM [3,14].

Stress caused by circumstances strongly affects ER or catecholamine levels in the brain [10–12]. A naturally gregarious mouse might suffer death by isolation. It has been reported that anesthetic sensitivity differs with the species of experimental animal or with sex [15].

In conclusion, we found that isolation influences enflurane sensitivity, causing an increase of about 1.2–1.3 times in enflurane requirements. Although it is already known that acute stress, like stimulation, affects ER in mice, we must determine the change in ER by chronic stress. It is necessary to pay attention to the population density in a cage when measuring anesthetic sensitivity. Researchers should use grouped mice, because isolated mice are not physiologically normal.

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