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Murine auto- and cross-tolerance to volatile anaesthetics

Auto-tolerance and cross-tolerance to halothane, isoflurane and enflurane were tested on 36 mice divided into three equal groups. Each group was first exposed to increasing concentrations of either of the three anaesthetics on 13 occasions. The concentration at which each mouse lost its righting reflex during successive exposures in a rotating cage was noted. Cross-tolerance was assessed by comparing the number of mice which had lost their righting reflexes during their first exposure to a given anaesthetic agent to the number which lost it after having been exposed to another anaesthetic.

All animals developed auto-tolerance to halothane, isoflurane and enflurane. Cross-tolerance was noted only between mice exposed to isoflurane and enflurane and between mice exposed to halothane and subsequently anaesthetized with isoflurane, but not vice versa.

Key words

ANAESTHETICS, VOLATILE: halothane, isoflurane, enflurane; POTENCY, ANAESTHETIC: tolerance.

Auto-tolerance is the inability to produce sleep with a previously effective concentration of an anaesthetic following several exposures to that anaesthetic. Cross-tolerance is the inability to produce sleep with a previously effective concentration of a given anaesthetic after numerous exposures to another anaesthetic.

Auto-tolerance or cross-tolerance to volatile anaesthetic may occur clinically in patients repeatedly exposed to one or more anaesthetic agents, especially severely burnt patients. Smith *et al.*¹ studied rapidly developing auto-tolerance in mice exposed to nitrous oxide, cyclopropane, nitrous oxide and helium, or ethylene and helium. They found that only animals exposed to nitrous oxide alone or to ethylene and helium developed auto-tolerance to these anaesthetics. In another study,² they discovered that mice exposed to 50 per cent nitrous oxide for two to three weeks became tolerant to that anaesthetic. When a second anaesthetic was added (cyclopropane or isoflurane), cross-tolerance to that anaesthetic was also noted. However, isoflurane when administered alone failed to produce auto-tolerance or cross-tolerance to other anaesthetics. After three weeks' exposure to nitrous oxide, 60 per cent of the animals convulsed after exposure, when held by the tail. Similarly, 43 per cent of mice convulsed when removed from 0.15 per cent isoflurane after a three-week exposure but only four per cent convulsed after six to nine weeks' exposure to the same concentration.

We decided to assess whether multiple short exposures to commonly used volatile anaesthetics produces auto-tolerance and cross-tolerance in mice. Clinical studies are recommended to assess the reproducibility of our results in man. This would permit the use of optimal sequential anaesthetic combinations in patients undergoing multiple anaesthesias and the use of minimal anaesthetic concentrations.

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FIGURE 1 System used to administer anaesthesia to mice in rotating cage. EM = electric motor, GB = gear box, P1 and P2 = pulleys transmitting the drive to the rotating cage placed in a transparent compartment, FGI = fresh gas inflow, EMMA = Engström Emma vapor analyzer, TO WM = to water manometer, M = mouse in rotating cage, L = barium hydroxide lime U.S.P., SS = scavenger system, EV = evacuation valve. The direction of drive of the system was reversible.

Methods

Eighteen Swiss Webster mice and 18 brown dominant mice (Jackson Laboratories) were divided into three equal groups. Each group included six white mice and six brown mice, randomly selected from the total group. One group (A) was exposed to halothane, the second group (B) was exposed to isoflurane, and the third group (C) to enflurane. All anaesthetics were delivered in oxygen at a flow rate of 5 L/min. The compartments in which the mice were successively exposed included two transparent containers. The first, or *static* container (capacity 5L) received gases from an anaesthesia machine. Excess gas was vented to atmosphere through a valved scavenger system. The floor of the chamber contained a perforated plate sandwiched between a layer of barium hydroxide lime, U.S.P. (1 Kg) below and a layer of straw above. The concentration of each volatile anaesthetic delivered by temperature compensated vaporizers was verified using an Emma analyzer (Engström, Sweden). The analyzer was placed on the line joining the common anaesthetic outlet of the anaesthesia machine to the chamber containing the mice. Chamber pressure

was measured on a water manometer to detect potential occult blockage of the scavenger system. The second, or *dynamic* chamber (Fig. 1), was similar to the first but contained a cylindrical cage, rotated by an electric motor three times per minute in either direction. At the end of all experiments, anaesthetic concentration was also measured on the scavenger side of both containers.

Experiments were first conducted in the rotating cage to assess the anaesthetic concentration at which each mouse lost its righting reflex, at which time it was considered to be asleep. Further exposures on two consecutive days were conducted in the static container. Mice were then retested in the dynamic container. Thus, all animals underwent 13 exposures to their allotted anaesthetic (five of them in the rotating cage). The initial anaesthetic concentration was increased by 25 per cent every 15 minutes and the number of mice which lost their RR at a given anaesthetic concentration (i.e. when they rolled passively on their backs on three consecutive occasions) was noted. Also noted was the condition of the animals before they lost their RR (excited, convulsing, vascillating on their legs or sleepy) and

the time taken for mice to lose their RR at each given anaesthetic concentration. In the static container, the animals of each group were exposed for 30 minutes to an anaesthetic concentration 25 per cent over that at which the most resistant mice had lost their RR in the rotating cage. When significantly fewer mice in a group lost their RR at a given concentration than that noted during the first exposure, auto-tolerance was considered to have obtained.

Group A and B mice (12 mice each) were originally submitted to 0.5 per cent halothane and isoflurane respectively. The 12 group C mice were initially given 0.75 per cent enflurane. Cross-tolerance was assessed by randomly dividing each group, which had undergone 13 exposures, into two subgroups of six mice each (three white and three brown mice). There were, thus, two halothane subgroups (A1 and A2), two isoflurane subgroups (B1 and B2), and 2 enflurane subgroups (C1 and C2). The mice in each subgroup were then exposed to one of the other anaesthetics. The number of mice in each group which had lost their RR at a certain anaesthetic concentration during the first exposure was compared to the number of animals in the subgroup which lost their RR when exposed to the same concentration. If the proportion in the subgroup was significantly smaller than that in the group, cross-tolerance was considered to have developed. The performance of white mice was also compared to that of brown mice.

Statistical significance was assessed by the chi square method using the Yates correction factor for small numbers. Results were compared, two at a time, using fourfold tables (i.e. number of mice asleep and number of mice awake in one group versus number awake and number asleep in the same group after further exposure to anaesthesia "auto-tolerance", or versus a subgroup "cross-tolerance"). The chi square value was used to obtain P from tables, at one degree of freedom. ($DF = \text{rows} - 1 \times \text{columns} - 1$, or $2 - 1 \times 2 - 1 = 1$). Values of $P < 0.05$ were considered significant.

Results

All mice receiving enflurane (group C) convulsed during anaesthesia. No convulsions occurred in the recovery period. Within a few minutes of exposure to all anaesthetics, mice became excited. As time progressed and the anaesthetic concentration was

raised, they vascillated on their legs from side to side, fell but righted themselves and finally lost their RR. Initially, they lost their RR within five minutes of exposure to a given anaesthetic concentration. As the number of exposures increased, so did the time needed for the mice to lose their RR. When they kept their RR over 12 minutes at any concentration, they lost their RR at the next higher concentration during the next exposure in the rotating cage. The tolerance of brown and white mice was not statistically significantly different. *Auto-tolerance to halothane* (group A) became significant during the seventh exposure (12 mice asleep on first exposure to 1 per cent halothane and only seven on the seventh exposure, $P < 0.05$, Table I). *Auto-tolerance to 1 per cent isoflurane* (group B) occurred on the tenth exposure (11 asleep during the first exposure and only five during the tenth, $P < 0.05$). *Auto-tolerance to 1.25 per cent enflurane* (group C) occurred on the seventh exposure (ten mice asleep during the first exposure and only three asleep during the seventh, $P < 0.025$) and to 1.5 per cent enflurane by the 13th exposure (12 mice asleep during the first exposure and only four by the 13th, $P < 0.005$).

Cross-tolerance was found when group A1 (six mice or half the halothane group) was exposed to isoflurane; two of six mice lost RR above one per cent compared to 11 of 12 mice in group B ($P < 0.005$, Fig. 2). When half the enflurane mice (the six mice in group C1) were exposed to isoflurane, 2 of 6 mice in group C1 and 11 of 12 in group B lost RR above one per cent ($P < 0.005$). When half the isoflurane mice (the six mice in group B1) were exposed to enflurane, two of six mice in group B1 and ten of 12 in group C lost their RR above 1.25 per cent ($P < 0.01$). No cross-tolerance was found when group A2 mice were exposed to enflurane, when group C2 mice were exposed to halothane or when group B2 mice were exposed to halothane.

Discussion

Our results suggest the possible existence of clinical auto-tolerance and cross-tolerance to volatile anaesthetics. Unlike other workers,¹⁻³ we did not use the anaesthetic dose 50 (anaesthetics concentration at which 50 per cent of mice lost their RR) to express auto-tolerance and cross-tolerance. This is because we wanted all groups studied to be submitted to the same number of exposures to each anaesthetic, and

TABLE I Loss of righting reflex

Anaesthetic	Exposure					
	Per cent	First	Fourth	Seventh	Tenth	Thirteenth
Group A (halothane)	1.0	12	10	7*	1	1
	1.25	—	2	5	10	10
	1.5	—	—	—	1	1
Group B (isoflurane)	0.75	2	—	—	—	—
	1.0	9	9	7	5*	4
	1.25	1	3	5	7	8
	1.5	—	—	—	—	—
Group C (enflurane)	1.0	2	2	—	—	—
	1.25	8	9	3†	1	1
	1.5	2	2	6	8	3‡
	1.75	—	1	3	3	8

Number of mice which initially lost their righting reflexes (RR) at each anaesthetic concentration, as the number of exposures was increased. All anaesthetics were delivered in oxygen at a flow rate of 5 L/min. Groups A and B mice were originally submitted to 0.5 per cent halothane and isoflurane respectively. Group C mice were initially given 0.75 per cent enflurane. N = 12 in each group. * = $P < 0.05$, † = $P < 0.025$, ‡ = $P < 0.005$.

to avoid differences in exposures when cross-tolerance was studied. For the same reason, enflurane, which has a substantially higher MAC (1.68) than both halothane (0.76) and isoflurane (1.15), was initially given at a concentration of 0.75 per cent and not 0.5 per cent as was used for the other two agents. However, since the highest concentration of halothane or isoflurane used was 1.5 per cent (Table 1), and that of enflurane 1.75 per cent, all groups were submitted to five different anaesthetic concentrations, each lasting 15 minutes.

The two-way cross-tolerance between isoflurane and enflurane, and enflurane and isoflurane (Fig. 2) was presumably due to their similar molecular configuration. Both are three carbon halogenated ethers with five fluorides and one chloride (the chloride in isoflurane being on carbon two and in enflurane on the first carbon). The lack of cross-tolerance between halothane and enflurane and enflurane and halothane was probably due to their different chemical compositions (halothane being a halogenated ethane and enflurane a three carbon halogenated ether). Although halothane has a higher lipid solubility than enflurane, the latter has convulsive properties which affect the cerebral neurons differently, because sleep and convulsions are closely related. It is well known that epileptics sleep for prolonged periods after a grand mal seizure. This may explain the lack of cross-

FIGURE 2 Diagram indicating the presence or absence of cross-tolerance among the three volatile anaesthetics studied. NS = no significant tolerance in the direction of the arrow. P values indicate cross-tolerance in the direction of the arrows.

tolerance between halothane and enflurane and vice versa. The cross-tolerance from halothane to isoflurane (but not from isoflurane to halothane) is probably due to the higher lipid solubility of halothane and because isoflurane, unlike enflurane, is not epileptogenic in clinical concentrations.

In addition, enzyme induction by anaesthetics, which is well known to raise MAC and, therefore, enhance tolerance, may have affected our results. Van Dyke *et al.*⁴ exposed 12 rats to 1 per cent

halothane and 12 rats to 1 per cent isoflurane for two hours. They measured ornithine decarboxylase induction by these agents as a function of pmol $\text{CO}_2/30 \text{ min/mg}$ liver protein. Halothane produced 13 ± 3.6 and isoflurane 22.7 ± 3.2 ($P < 0.025$). This may explain the tolerance to isoflurane of mice exposed to halothane, but not vice versa.

Clinical studies should be conducted on patients receiving multiple anaesthesias, and, thus, assess the potential development of auto-tolerance and cross-tolerance with different volatile agents. This will permit the use of the optimal anaesthetic sequential combination and result in the administration of minimal doses.

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Résumé

L'auto-tolérance et l'inter-tolérance à l'halothane, l'isoflurane et l'enflurane furent étudiées chez 36 souris, divisées en trois groupes égaux. Chaque groupe fut d'abord exposé à des concentrations croissantes de chacun des trois anesthésiques à treize reprises. La concentration à laquelle chaque souris perdit son réflexe de redressement fut notée. Le développement de l'inter-tolérance fut obtenu en comparant le nombre de souris ayant perdu leur réflexe de redressement durant la première exposition à un anesthésique particulier au nombre l'ayant perdu après exposition à un autre anesthésique. Tous les animaux développèrent une auto-tolérance à l'halothane, l'isoflurane et l'enflurane. L'inter-tolérance fut découverte seulement chez les souris exposées à l'isoflurane et l'enflurane et chez celles exposées d'abord à l'halothane et ensuite à l'isoflurane, mais non pas vice versa.