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Inhibitory action of halothane on rat masculine sexual behavior and sperm motility

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Abstract

Adult male rats were exposed to inhale halothane in the following regime: 15 ppm/4 h/5 days/week/9 weeks. Sexual behavior observations and sperm motility test were made before halothane exposure (0 days) and at 15, 30, 45 and 60 days of exposure. Fifteen days after halothane exposure, this anesthetic inhibited the proportion of animals displaying ejaculation. In those animals ejaculating, halothane produced an inhibition of masculine sexual behavior reflected as an increase in the intromission latency, number of mounts and postejaculatory interval. At 30 days after exposure, only an increase in the intromission latency was observed. At 45 and 60 days, the inhibitory effect of halothane on sexual behavior disappeared. Similarly, at 15 and 30 days, but not at 45 or 60 days of halothane exposure, a reduced sperm motility was observed. Such transient effects of halothane suggest the development of tolerance to the inhibitory actions of this anesthetic on sexual behavior and sperm motility. These halothane effects are in line with an inhibition of masculine sexual behavior after stimulation of the GABAergic system.

Keywords: Halothane; Male sexual behavior; GABA; Sperm motility

1. Introduction

Several epidemiological reports have suggested that there is, among female anesthesiologists, hospital operating room nurses and wives of male anesthesiologists, a greater than normal incidence of spontaneous abortions (Askrong and Harvald, 1970; Cohen et al., 1971, 1974; Vaisman, 1967). Furthermore, Knill-Jones et al. (1972) reported an abnormal high incidence of infertility in anesthesiologists and of congenital abnormalities in the offspring of those conceiving. A common denominator in all these reports is that the operating room personnel are repeatedly exposed to low levels of volatile anesthetics in the course of their daily occupation. The most commonly used anesthetic gas is halothane, which is the suspected toxic agent causing the reproductive alterations (Sturrock and Nunn, 1976).

Animal studies using low levels of halothane have not consistently showed damaged on reproductive functions. Thus, Doenicke et al. (1975) exposed male rats to 8000 ppm/4 h/day/9 weeks and found a very low birth rate in their offspring, whereas Wharton et al. (1978) reported that exposure of mice to subanesthetic concentrations of halothane (3000 ppm/4 h/17 weeks) does not result in important abnormalities in their offspring. These data suggest that there may be a dose-dependent response and/or a species difference.

There are scarce reports that specifically studied the effects of general anesthetics at low concentrations on sexual behavior. In 1988, Gillman showed that the anesthetic compound, nitrous oxide, at analgesic concentrations, caused a depressant effect on an objective measure of masculine sexual behavior, such as erection, in most male human subjects studied. Male sexual behavior is integrated in the central nervous system through the interaction of excitatory and inhibitory neurotransmitters. We have demonstrated that GABA (the main inhibitory neurotransmitter in the mammalian brain), via $GABA_A$ receptors, inhibits sexual arousal

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and mediates the sexual inhibition that follows ejaculation (Fernández-Guasti et al., 1986a,b). On the other hand, it has been reported that halothane-induced anesthesia depends on the interaction of this drug with $GABA_A$ receptors (Jones et al., 1992; Lin et al., 1992). Taken together, these data suggest that halothane may alter male reproductive function and cause its anesthetic action by a common mechanism.

The present study was designed to examine the changes in the male rat reproductive function accompanying subchronic halothane exposure. We included the analysis of masculine sexual behavior as a reflection of the actions of this anaesthetic in the central nervous system and sperm motility as a measurement of semen quality.

2. Materials and methods

2.1. Animals

Female and male Wistar rats (8 weeks of age) were used. Animals were housed in wired hanging steel cages in groups of five animals and were allowed filtered water (Water Quality, Mexico) and rat chow standard diet (PMI Feed, St. Louis, MO) ad libitum. Rats were kept at 25 °C and 50% relative humidity and under a 12-h dark/12-h light cycle (starting at 07:00 h). Only sexually active males were used in this study. Males were considered sexually active if they ejaculated in at least two sexual behavior tests (see below for details on the sexual behavior tests). Forty males were tested for sexual activity from which 27 fulfilled the criterion of sexually active and were divided in two groups.

2.2. Halothane exposure

The treated group ($n=15$) was exposed to halothane (Zeneca, UK) (15 ± 1.5 ppm/4 h/day/5 days/week/9 weeks) and the control group ($n=12$) received air. The time of exposure was selected as recommended by Wolff and Dorato (1997). The halothane dose was selected considering that the level of the anesthetic in surgery theatres ranges from 11 to 63 ppm (Bruce and Bach, 1976; Dossing and Weihe, 1982; Karelova et al., 1992; Smith and Shirley, 1978). Five rats were placed in each glass inhalation chamber. The experiment was conducted in compliance with the Mexican Regulations of Animal Care and Maintenance (NOM, 2001).

Inhalation exposure to either halothane or air was performed simultaneously in different glass chambers. Halothane was delivered by a dispensing pump (B. Braun, Melsurgen, Germany) set at a constant flow rate of 1.5 ml/h to a round-bottom boiling flask placed on a heating mantle to evaporate halothane (boiling point, 53 °C). The concentration of halothane (15 ± 1.5 ppm) was achieved within 10–15 min, using air as the carrier, dispensed into chambers using Tygon tubing, at a flow rate of 5 l/min. Control groups received air alone at the same flow. Soda lime was placed on the floor of the glass chambers to maintain the CO_2 level at 0.3%. These concentrations were monitored every hour using a Gow-Mac gas chromatographer equipped with a thermal conductivity detector model 580 (Bridgewater, NJ) coupled to a primary computing integration chromatography data system version 6.0 using a concentric column CTR1 (Altech, Germany). Concentrations of halothane were also monitored every hour using a gas chromatographer coupled to a mass spectrophotometer detector (Model GCQ, Finnigan MATT, San Jose, CA) and a PONA chromatography column of 50 cm length, 0.2 mm internal diameter and 0.5 μm film (Hewlett Packard, Palo Alto, CA). In addition, a standard curve was built using different halothane concentrations (5, 10, 15, 20 and 25 ppm) in a static chamber in order to establish the optimal analytical conditions for the environment of the dynamic glass chambers. A similar temperature (25 °C) existed in the experimental chambers and the environment. According to good laboratory practices for inhalation toxicological studies, the weight of animals should not exceed 10% of the chamber internal total space (Riley and Conning, 1990). This criterion was fulfilled throughout the present experiment.

2.3. Sexual behavior tests

The mating tests were performed according to Fernández-Guasti et al. (1986a). The sexual behavior tests were con-

Table 1
Effect of halothane subchronic inhalatory exposure on the proportion of animals showing masculine sexual behavior

	Days									
	0		15		30		45		60	
	C	H	C	H	C	H	C	H	C	H
Mounts (n'/n)	12/12 (100%)	15/15 (100%)	10/12 (83%)	12/15 (80%)	11/12 (92%)	13/15 (87%)	12/12 (100%)	14/15 (93%)	12/12 (100%)	13/15 (87%)
Intromissions (n'/n)	12/12 (100%)	15/15 (100%)	10/12 (83%)	12/15 (80%)	11/12 (92%)	12/15 (80%)	12/12 (100%)	14/15 (93%)	12/12 (100%)	13/15 (87%)
Ejaculators (n'/n)	12/12 (100%)	15/15 (100%)	9/12 (75%)	8/15* (53%)	11/12 (92%)	11/15 (73%)	12/12 (100%)	14/15 (93%)	12/12 (100%)	12/15 (80%)

Animals were observed for 30 min at 0, 15, 30, 45 and 60 days after halothane subchronic inhalatory exposure. n': animals that showed sexual behavior; n: total number of animals.

* MacNemar test, $P<.05$.

ducted under red dim light at the beginning of the dark phase of the 12-h light/12-h dark controlled cycle. The observations were made in Plexiglas cages (44×54×21 cm). Sexually active males (which ejaculated at least twice in three consecutive sexual behavior tests separated by a week interval) were placed in separated cages for 5 min; thereafter, a sexually receptive female was introduced. Females were brought into sexual receptivity by the sequential treatment with estradiol benzoate, 4 μg/rat (Sigma, St. Louis, MO), and 44 h later with progesterone, 2 mg/rat (Sigma), injected 4 h before testing. Each test lasted for 30 min or once a copulatory series was completed. A copulatory series included the events from the first intromission to the end of the postejaculatory interval (the first intromission of the

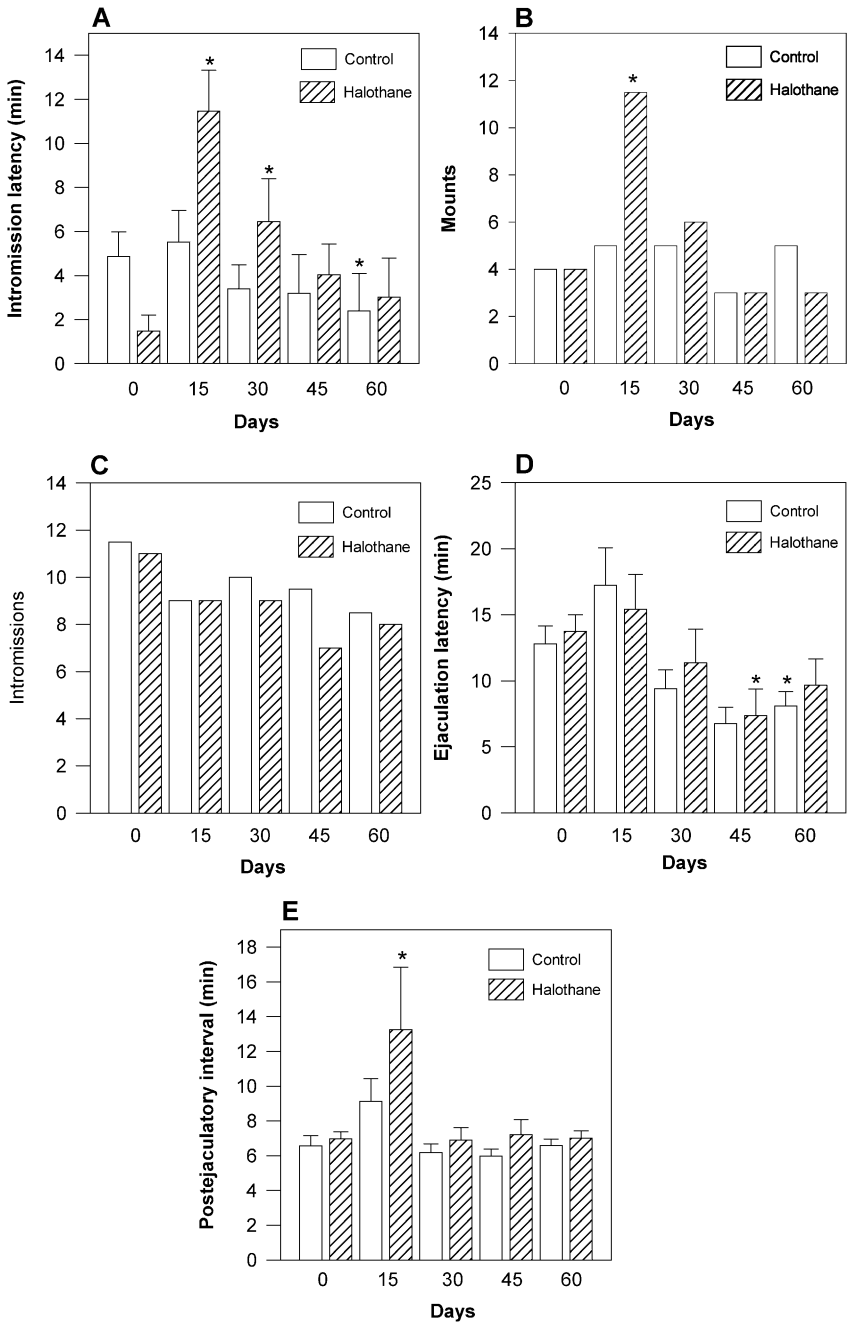

Fig. 1. Effect of halothane subchronic inhalatory exposure on the sexual behavior of male rats. (A) Intromission latency, Friedman ANOVA $H=11.5$, $P<.02$ for controls and $H=25.6$, $P<.0001$ for halothane-treated rats; (B) mounts, Friedman ANOVA $H=7.0$, n.s. for controls and $H=18.7$, $P<.001$ for halothane-treated rats; (C) intromissions, Friedman ANOVA $H=4.41$, n.s. for controls and $H=7.01$, n.s. for halothane-treated rats; (D) ejaculation latency, Friedman ANOVA $H=10.9$, $P<.05$ for controls and $H=11.4$, $P<.02$ for halothane-treated rats; and (E) postejaculatory interval, Friedman ANOVA $H=4.54$, n.s. for controls and $H=13.9$, $P<.02$ for halothane treated. Data were calculated from those animals that ejaculated (for n, see Table 1) at 15, 30, 45 or 60 days and their respective control value before air or halothane exposure (0 days). Wilcoxon t test versus control nonexposed values (0 days), $^*P<.01$.

second ejaculatory series). The number of animals showing mounts, intromissions and ejaculation was recorded in each test. In those males ejaculating, the following parameters were registered: (a) intromission latency; (b) number of mounts; (c) number of intromissions; (d) ejaculation latency; and (e) postejaculatory interval (for definition of each parameter, see Fernández-Guasti et al., 1986a). Sexual behavior tests were made before (0 days) and at 15, 30, 45 and 60 days of halothane or air exposures.

2.4. Sperm motility

In the sexual behavior tests performed at 0, 15, 30, 45 or 60 days of halothane or air exposures, immediately after ejaculation, a 1-ml saline vaginal wash was taken from the female receiving the ejaculate, and the semen suspension was deposited in a prewarmed test tube. A 15-μl aliquot of the semen suspension was placed in a glass slide prewarmed and maintained at 37 °C (Slide Warmer, Model 77, Fisher Scientific, Pittsburgh, PA). The sample was analyzed by light microscopy within 30 min after ejaculation under 40× magnification using a microscope Olympus BX40 (Olympus, Melville, NY), and 8–10 fields were observed to complete 100 cells. From this number, the percentage of motile cells was estimated (Seed et al., 1996).

2.5. Statistical analysis

Results represent the means ± standard errors or standard deviations for sexual behavior or sperm mobility, respectively. The statistical analysis was made within the halothane or air exposed groups by comparing the data obtained before the treatment (0 days) and those collected at 15, 30, 45 and 60 days after exposure. The proportion of copulating subjects was compared using the McNemars test. For the sexual behavior specific parameters and for sperm motility, a Friedman analysis of variance was followed by a Wilcoxon *t* test. For these analyses, the comparisons included those data from animals that ejaculated after exposure to halothane or air at 15, 30, 45 or 60 days versus their respective control values at 0 day. At the end of the experiment (60 days), the animals in both groups were weighed, and the data were compared using the Mann–Whitney *U* test.

3. Results

3.1. Halothane effect on body weight

Subchronic exposure to halothane for 60 days did not affect the body weight of animals. At the end of the experiment, the exposed group had a mean ± S.D. of 440.96 ± 8.16 g, and for the control animals, it was 454.45 ± 13.40 g. No statistical differences were found (Mann–Whitney *U* test).

3.2. Halothane effects on rat male sexual behavior

The sexual behavior of halothane-exposed animals is shown in Table 1 and Fig. 1. Table 1 exhibits the proportion of animals showing mounts, intromissions and ejaculation in a 30-min test at different exposure intervals: before halothane exposure (0 days) and at 15, 30, 45 and 60 days after subchronic exposure to either the anesthetic or air. Clearly, 15 days after subchronic exposure to halothane, a reduction in ejaculating animals was found, but no significant decreases in the animals showing mounts or intromissions were observed at this time. At 30 days of halothane exposure, the tendency of a reduced number of ejaculating animals was found, although it did not reach statistical significance. At 45 or 60 days of halothane exposure, no effects were seen. In the control group exposed to air, no statistical significant differences were found in the proportion of males showing sexual behavior along the experiment.

Fig. 1 shows the parameters of masculine sexual behavior after subchronic exposure to air or halothane. At 15 days, an increase in the intromission latency, number of mounts and postejaculatory interval were found in copulating animals. At 30 days after halothane exposure, only an increase in the intromission latency was observed. At 45 and 60 days, no effects of halothane exposure were found, except for a slight reduction in the ejaculation latency, in both halothane and control groups. This could be due to the constant animal sexual behavior training.

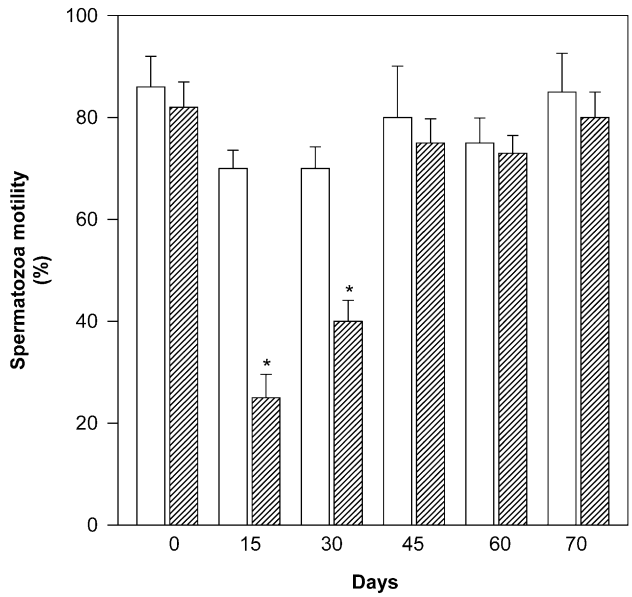

Fig. 2. Effect of halothane subchronic inhalatory exposure on sperm motility. Friedman ANOVA $H = 7.71$, n.s. for controls and $H = 22.5$, $P < .001$ for halothane-treated rats. Data were calculated from those animals that ejaculated (for *n*, see Table 1) at 15, 30, 45 or 60 days and their respective control value before air or halothane exposure (0 days). Wilcoxon *t* test versus control nonexposed values (0 day), $^*P < .01$.

3.3. Halothane effect on sperm motility

The percentage of sperm motility in animals subchronically exposed to halothane or air is shown in Fig. 2. At 15 and 30 days of halothane exposure, sperm motility was clearly reduced (29% and 42.5%, respectively). However, it recovered at 45 and 60 days after treatment. In the air-exposed group, no statistical significant differences were found.

4. Discussion

Halothane exposure resulted in an inhibition of sexual behavior and sperm motility after 15 days, and to a lesser extent after 30 days of subchronic exposure. Thereafter, at 45 or 60 days, all halothane actions disappeared. The inhibitory actions of halothane on the percentage of rats ejaculating (at 15 day) was specifically accompanied by a drastic increase in the intromission latency, postejaculatory interval and number of mounts and an important decrease in sperm motility in those animals copulating. Control (air exposed) rats at 15 day showed values of these parameters, similar to those registered before exposure (0 day).

The inhibition of sexual behavior produced by halothane was primarily observed as a reduction in the number of ejaculating animals and, in those copulating, as prolonged intromission latency and postejaculatory interval and increased number of mounts before ejaculating. Such inhibition is consistent with the GABAergic action of this anaesthetic: halothane causes its anesthetic effect through the $GABA_A$ receptor, by enhancing the Cl^- currents (Jones et al., 1992; Lin et al., 1992). It has been reported that GABA agonists, for example, muscimol, or compounds that prevent GABA breakdown, as ethanolamine-*O*-sulphate, inhibit masculine sexual behavior evidenced by a drastic decrease in the percentage of copulating animals. Interestingly, GABA infusion into the medial preoptic area, a brain structure essential for the expression of masculine sexual behavior, produces a prolonged intromission latency (Fernández-Guasti et al., 1986a). Conversely, GABA antagonists such as picrotoxin and bicuculline or inhibitors of GABA synthesis drastically facilitate sexual behavior primarily by shortening the postejaculatory interval and the emission of ultrasonic vocalizations that characterizes this period (Fernández-Guasti, et al., 1986b).

Present results, showing transient inhibitory effects of halothane at 15 and 30 days but not at 45 or 60 days of exposure, suggest the development of tolerance to the actions of this anesthetic. Drug tolerance may develop after an increase in the metabolic rate of a compound (O'Brien et al., 1996). Our group has recently demonstrated an increased activity of the cytochrome CYP2E1 (the principal metabolizing enzyme of halothane) after the subchronic exposure to halothane in several tissues. Additionally, we have found an increased interaction of trifluoroacetyl halide, the main halothane oxidative metabolite, with microsomal proteins at 60 days after exposure (submitted manuscript). These findings suggest that the increased metabolism of the anesthetic could account, at least partly, for the transient actions observed in the present study.

On the other hand, it has also been reported that the prolonged daily inhalation of halothane produces tolerance, evidenced as fewer acoustic evoked responses in brain areas closely related to the control of masculine sexual behavior (Fuller et al., 1985). Moreover, Nakahiro et al. (1989) reported a similar tolerant effect to GABA in neurons chronically exposed to this neurotransmitter. Finally, the dose–response curve to isoguvacine, a $GABA_A$ agonist, shifted to the right in neurons chronically exposed to GABA. Taken together, these data suggest that the inhibitory actions of halothane on sexual behavior are mediated after the stimulation of the GABAergic system and may also explain the lack of action of this anesthetic after prolonged exposures. Whether tolerance develops to the inhibitory effects of various GABA agonists on masculine sexual behavior remains to be established.

Interestingly, halothane also reduced the sperm motility after daily exposure at 15 and 30 days. Hinkley (1979) found that halothane exposure in vitro reversibly inhibits the motility of sea urchin sperm dose-dependently at concentrations up to 5 mM. On the other hand, it has been recently demonstrated that both $GABA_A$ and $GABA_B$ receptors are present in sperm cells (Calogero et al., 1999; He et al., 2001). In addition, it has been shown that GABA, mainly by activation of the $GABA_A$ receptor, modulates sperm motility and increases hyperactivation and acrosome reaction at the same extent than progesterone, suggesting that GABA has a role in sperm function and activation (Calogero et al., 1996). From these data, it might be suggested that halothane acts through a similar mechanism to inhibit sperm motility and male sexual behavior.

Although the halothane dose (15 ppm) used in this study is within the ambient levels (11–63 ppm) reported for operating rooms (Linde and Bruce, 1969; Whichter et al., 1971), the exposure regimen and the administration of a single anesthetic agent, represent different situations from those encountered by the operating team. Therefore, considerable caution is necessary when comparing experimental laboratory studies of subchronic exposure to halothane with the clinical situation.

In summary, the present study shows for the first time that a subchronic halothane exposure specifically inhibits male sexual behavior and sperm motility. These halothane effects seem to be mediated by the GABAergic system. Such effects were transient at 15 days of daily halothane exposure and disappeared after 1 month, suggesting the development of tolerance. In general, our results encourage further research to evaluate the reproductive function that may be compromised in humans chronically exposed to general anesthetics.

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