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The Effect of Halothane on Mice Selectively Bred for Differential Sensitivity to Alcohol[1]

RODNEY BAKER, CHRISTINE MELCHIOR[2] AND RICHARD DEITRICH

University of Colorado School of Medicine, Department of Pharmacology
4200 E. 9th Avenue, Denver, CO 80262

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BAKER, R., C. MELCHIOR AND R. DEITRICH. *The effect of halothane on mice selectively bred for differential sensitivity to alcohol.* PHARMAC. BIOCHEM. BEHAV. 12(5) 691–695, 1980.—We have investigated the response of mice to halothane that have been selectively bred for either sensitivity (long sleep, LS) or resistance (short sleep, SS) to the anesthetic effects of ethanol. While large differences in the response of the animals to ethanol were observed in sleep time, blood anesthetic concentration at time of awakening, and body temperature, the SS and LS lines did not differ in their response to halothane. We conclude that the mechanism of action of ethanol and halothane differ in a significant way from each other.

Halothane Ethanol Selective breeding

THE mechanism by which ethanol elicits its hypnotic action is unknown. The hypnotic effects of ethanol as well as other aliphatic alcohols appear to be closely related to the action of gaseous anesthetics, in that an interaction between the anesthetic and a unique receptor has not been shown. However, it has been suggested that the compounds evoke a common alteration, and that the relative potency of the compound is determined by its concentration in neuronal cell membranes [26]. Such a working hypothesis is based primarily on the observation that the relative potency of various anesthetic agents, including short chain aliphatic alcohols, correlates well with their oil-water partition coefficients [10,26]. The original observations of Overton [22,23] and Meyer [20] have subsequently been modified to include the thermodynamic action activity and molar volume of the anesthetic compound [5,21]. If these physical properties are included, a very good correlation exists between these properties and anesthetic potency.

The relationship between oil-water partition coefficients and hypnotic potency suggests that perturbation of membrane function may be mediated through changes in the lipid portion of neuronal membranes. The presence of the compound may alter lipid-lipid or lipid-protein interactions, however, the assumption is that the presence of the compound in the membrane is primarily responsible for its action and a unique receptor or interaction is not required for anesthetic action. The possibility of specific receptors being involved in the mechanism of anesthetic action is unlikely considering the range of structures of compounds which have anesthetic properties and the high doses which are required. It has been suggested, however that specific compounds may interact with distinct proteins or sites on membrane proteins which result in conduction blocks [24].

An unique opportunity exists to investigate the relationship between the anesthetic actions of the gaseous anesthetics and ethanol. Mice have been selectively bred for sensitivity (long sleep, LS) or resistance (short sleep, SS) to the acute actions of ethanol [17]. The sensitivity is apparently centrally mediated since the rates of alcohol metabolism are identical in the two lines [11]. These animals are derived from a heterogeneous line of animals which was originally developed from an 8-way cross of inbred animals. The SS and LS animals are outbred and are selected only for their response to the acute CNS depressant effects of alcohol. In theory, any differences between these two lines should be related, to a greater or lesser degree, to the cause of the differential response to ethanol.

Previous studies indicated that the differential sensitivity to ethanol exhibited by LS and SS mice was shared by other alcohols, but not by other CNS depressants such as pentobarbital [4]. Therefore, it was hypothesized, if SS and LS animals are differentially sensitive to a gaseous anesthetic such as halothane, it would be presumptive evidence that alcohols and gaseous anesthetics have similar if not actually identical mechanisms of action. Should this fail to be the case, however, it would be strong evidence that the actions of the two classes of agents differ to a significant degree.

METHOD

Materials

Two lines of mice (LS and SS) obtained from the Institute

[1]Supported by Grants AA-03527 Alcohol Research Center and DA-07043.
[2]NIDA Postdoctoral Fellow. Present address: Department of Physiology, University of Illinois, School of Medicine, Chicago, IL 60680.

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for Behavioral Genetics, Boulder, CO were used throughout the study. The animals were from the 26th generation and were tested at 60–80 days of age. Both sexes were used. Halothane, 2-bromo-2-chloro-1,1,1-triflouroethane which contained 0.01% thymol (w/w) was obtained from Ayerst Laboratories, Inc., New York, NY. Ethanol 95% (v/v) was obtained from U.S. Industrial Chemicals Co., New York, NY. The anesthetics were administered either by inhalation or IP injection, the specific method employed is given in the figure or table legends.

Response to ethanol or halothane was measured by the period of time the animals were devoid of the righting reflex or the ability to remain on a vertical wire cylinder 20 cm in diameter and 10 cm in length, suspended 30–60 cm from the bottom of the cage.

If the anesthetic was administered by inhalation, the mice were placed in a 4 L jar. Halothane was introduced into the chamber as a 1–2% halothane, 98–99% air mixture at a rate of 1 L/min using a halothane metering device to control halothane concentration. Ethanol was introduced by bubbling air (5 L/min) through a 95% v/v ethanol solution which was maintained at 24°C. The long sleep and short sleep animals were exposed to gaseous halothane for 30 min and ethanol for 60 min.

Rectal body temperature was measured with a YSI model 44TA thermometer (Yellow Springs Instrument Co., Yellow Springs, OH). The rectal probe was lubricated with glycerol and inserted 1.5 cm into the rectum. Body temperature was determined 15 min prior to treatment and at 15 min intervals thereafter for 180 min. Animals used to determine the hypothermic effect of halothane or ethanol were not utilized to determine other parameters.

Blood samples were obtained from the retro-orbital sinus. Ethanol or halothane levels were measured by gas chromatography using a head space technique [3]

RESULTS

Figure 1 illustrates the dose response curves for halothane and ethanol. The parameter measured was the ability to remain on a vertical wire cylinder, percent responding corresponds to the animals which fell from the wire 3 times within 2 min. The response to ethanol was consistent with previous studies using the LS and SS mice in that a significant difference between LS and SS animals was observed. The ED_{50} values using ethanol were 1.7 g/kg and 2.8 g/kg for the LS and SS lines, respectively. Halothane was more potent, as measured by the same parameter. However, the two lines responded identically. The ED_{50} was 1.1 g/kg for both LS and SS mice.

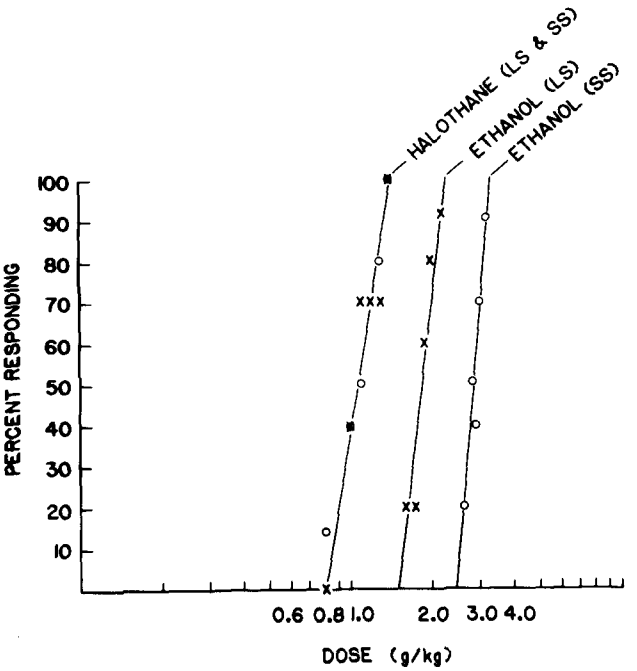

FIG. 1. Dose response curve for halothane and ethanol in long sleep and short sleep mice. Animals were treated IP. Percent responding indicates animals which were unable to remain on a 20 cm wire cylinder.

TABLE 1

EFFECT OF HALOTHANE AND ETHANOL ON RIGHTING REFLEX ON LONG AND SHORT SLEEP MICE

Line	n	Anesthetic*	Regain righting reflex† (sec) ± SEM	Blood anesthetic‡ concentrations mg/dl ± SEM
LS	(10)	Halothane (Exp 1)	373 ± 81.4	54.8 ± 4.7
SS	(10)	Halothane (Exp 1)	256 ± 57.1	54.9 ± 5.7
LS	(10)	Halothane (Exp 2)	420 ± 62.0	40.8 ± 4.1
SS	(10)	Halothane (Exp 2)	348 ± 54.0	46.5 ± 6.1
LS	(10)	Ethanol	7620 ± 1772.0§	240 ± 17¶
SS	(10)	Ethanol	1680 ± 360	426 ± 40

*Halothane administered as 2% halothane 98% air at a flow rate of 1 L/min. Ethanol supplied by passing air (5 L/min) through a 95% v/v ethanol solution which was maintained at 24°C. The animals were exposed to halothane for 30 min and to ethanol for 90 min. Experiments I and 2 are duplicates, carried out on different days.

†Time corresponds to period animals were devoid of righting reflex, 0 being the point at which exposure to each anesthetic was terminated. The plasma concentrations of halothane or ethanol were equal in the LS and SS lines at 0 time.

‡Blood anesthetic concentrations at time animals regained righting reflex.

§significantly different from SS ethanol $p<0.01$.

¶significantly different from SS ethanol $p<0.001$.

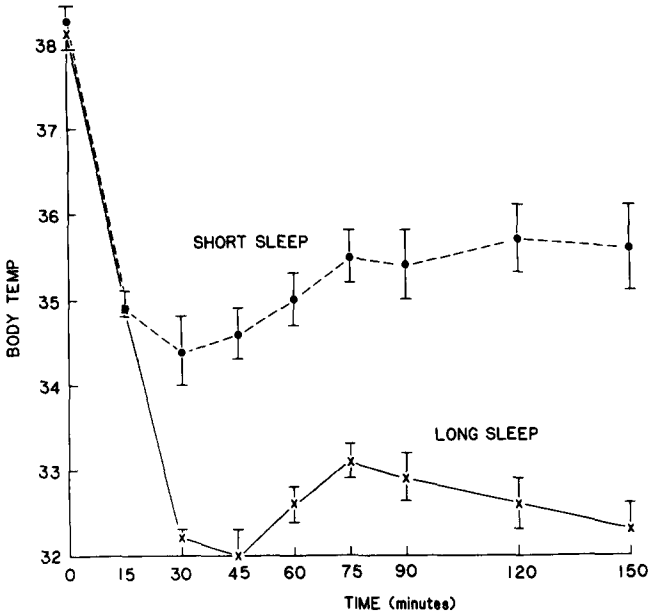

FIG. 2. Hypothermic effect of ethanol in long sleep and short sleep mice. Animals treated with 4.1 g/kg ethanol IP.

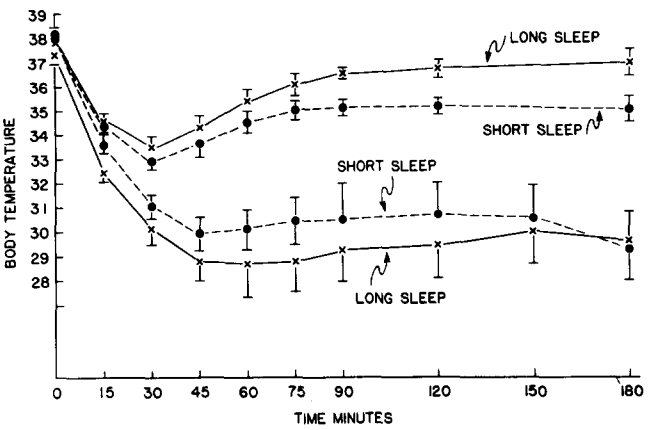

FIG. 3. Hypothermic effect of halothane in long and short sleep mice. Animals treated wtih 0.8 g/kg halothane upper curves, or 1.37 g/kg halothane lower curves.

In order to determine if the rates of absorption or elimination of ethanol or halothane were responsible for the observed effects, blood halothane and ethanol concentrations were measured in mice given the ED_{50} doses. Blood samples were taken at the time the animals fell from the wire or at 7 min after the injection, if the individual had not fallen. A significant difference in blood ethanol concentration was apparent between the LS and SS animals at the time the animals fell from the wire. Mean blood ethanol concentration (±SEM) of the LS mice at the time the animals fell was 192.0 ± 15.0 mg/dl (n=4). A value of 184.5 ± 17.3 (n=6) was determined for animals which remained on the wire for 7 min. Comparable values for SS mice were 290 mg/dl ± 13.5 (n=5) and 256 mg/dl ± 11.1 (n=5). The differences are significant at $p<0.01$ level. No significant difference in halothane concentration was found between SS and LS mice given the ED_{50} dose (1.1 g/kg). Blood halothane concentrations of 25.4 mg/dl ± 2.1 (n=5) and 22.0 mg/dl ± 2.4 (n=5) were found in responding and nonresponding LS mice. Responding SS mice had blood halothane concentrations of 27.5 mg/dl ± 2.1 (n=6). None of the differences are statistically significant.

The effect of ethanol and halothane administered by inhalation to LS and SS mice is given in Table 1. Response was measured by determining the period of time the animals were devoid of the righting reflex, using animals which had been exposed to halothane for 30 min or ethanol for 60 min. A mouse was considered awake when it was able to right itself 3 times within 1 min. No significant difference was measured between the two lines in their response to halothane, as determined by sleep time or blood halothane concentration at the time the animals regained the righting reflex. A significant difference in sleep time was observed between LS and SS animals exposed to ethanol vapor consistent with previous studies in which ethanol was given IP and ability to remain on a wire screen was measured. The period of time the LS animals were unable to right themselves was longer than that of SS, and SS mice had a higher blood ethanol concentration than LS at the time they regained the righting reflex. Halothane elimination rates following a 30 min exposure to 2% halothane 98% air mixture were not significantly different between LS and SS animals.

The divergence in response to ethanol or halothane by the LS and SS lines was also maintained with regard to the hypothermic effect of the two anesthetics. The hypothermic action of ethanol is greater in the LS animals than in the SS mice ([13], Fig. 2). However, the depression of body temperature by halothane was not different in the two lines (Fig. 3). The SS line displayed a somewhat greater sensitivity to a 0.08 g/kg dose of halothane. Whereas the hypothermic effect of a 1.37 g/kg dose was slightly more pronounced in the LS line, we conclude that no marked differences exist between the two lines with respect to the effect of halothane on body temperature.

DISCUSSION

Recent theories proposed to explain the mechanism of general anesthetic action have generally been divided on the basis of a direct or indirect interaction of anesthetic and the membrane proteins involved in neuronal function. The explanation of anesthetic action by direct interaction is that membrane proteins are rendered incapable of normal function through binding of the anesthetic to the protein component of the membrane [9, 18, 24, 32]. The indirect mechanism is based on the anesthetic exerting its effect through the lipid phase. Alterations of membrane structure and phospholipid bilayers by various anesthetics, including short chain alcohols, has been demonstrated by various methods [2, 12, 16, 26].

The classical work of Overton [23] and Meyer [20] and much of the later work has correlated anesthetic potency with oil/water partition coefficients or thermodynamic properties of the anesthetics. The underlying assumption in relating anesthetic potency to lipid solubility appears to be that the lipid component of neuronal membranes is uniform throughout the membrane. A considerable amount of evidence exists, however, which indicates that membrane structural asymmetry is extensive and involves all components of the membrane. Phosphatidylethanolamine, and

phosphatidylserine appear to be located on the inner surface of erythocyte membranes whereas phosphatidylcholine and sphingomyelin are located in the outer surface [8,31]. Fluid and solid regions seem to coexist in mitochondrial membranes suggesting regional differences in membrane composition [14]. Although most studies relating to asymmetric composition of membrane lipids have not addressed neuronal tissue, asymmetric distribution of phosphatidylethanolamine and acyl groups of the phospholipid fraction has been demonstrated in synaptosomal fractions obtained from total mouse brain [6]. Results from x-ray diffraction studies also indicate asymmetric distribution of protein and lipid fractions of myelin bilayers [1]. A portion of membrane lipids are directly associated with proteins and it appears that specific proteins associate with a different array of phospholipids [16].

It has been demonstrated that halothane achieves equilibrium within phosphatidylcholine vesicles, and exchanges rapidly between aqueous and phospholipid compartments of the vesicles [28]. The authors concluded that the possibility of a stable phospholipid-anesthetic or water-anesthetic interaction was not consistent with their results. Trudell [29] suggested that anesthetics act by fluidizing nerve membranes such that fluid-solid phase separation required for neuronal function is compromised. If the primary anesthetic action of halothane or ethanol is related to the fluidizing effect of the compounds toward neuronal membranes, these results suggest that the anesthetic potency of ethanol and halothane is not only a function of the physical and chemical properties of the compounds, but also influenced by membrane characteristics.

Considering the complexity of biological membranes, it is not possible to conclude from this study that the anesthetic action of halothane or ethanol is due to perturbation of lipid components or direct binding to target membrane proteins. It appears, however, that the anesthetic action of halothane and ethanol is not due to incorporation of the compounds equally throughout total membrane lipids. If both compounds act by perturbing membrane lipid structure or physical state, the LS and SS lines must differ in membrane areas that have a high affinity for ethanol. Assuming that anesthetics generally act directly by binding to membrane proteins, halothane and ethanol must interact with separate proteins or lipophilic protein sites.

Although these results indicate that the mechanism responsible for alcohol anesthesia differs from that of halothane, it is possible that the differences are due to factors not related to the primary mechanism of anesthesia.

The hypothermic effect of ethyl alcohol is significantly greater in the LS line as compared to the SS, whereas halothane elicits an equal hypothermic effect on both lines. If anesthetics act by fluidizing neuronal cell membranes [2,29] a change in body temperature should influence the potency of an anesthetic. Comparing the LS and SS lines, however, the drop in body temperature and sleep time is greater in the LS lines, whereas a decrease in body temperature should antagonize the fluidizing effect of ethanol.

Many substances which cause CNS depression lower body temperature. However, this heat loss may simply reflect an inability to thermoregulate rather than an activation of heat loss pathways. With ethanol, it has been shown that the degree of hypothermia is dose-dependent, but can be prevented by elevating environmental temperature [7]. Since an injection of ethanol into the cerebral ventricle of mice produces a fall in temperature at a dose that has no effect when administered intraperitoneally [25], a central component is involved. The neurochemical system(s) underlying this response has yet to be clearly defined, although some evidence supports the view that a dopaminergic system is involved [27]. This study also suggests that the hypothermic effect of ethanol and its anesthetic effect may be related. However, using the heterogeneous stock from which the LS and SS mice were derived, a correlation between sleep time and change in body temperature could not be demonstrated (Eriksson, personal communication).

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