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Hypothermia decreases ethanol MAC in rats

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Abstract
Despite the known capacity of hypothermia to increase anesthetic potency (decrease the partial pressure required to produce anesthesia), many in vitro studies examine the effects of ethanol and other anesthetics in oocytes or isolated neurons at room temperature. We tested whether, as predicted for potent inhaled anesthetics, a proportionate increase in solubility with hypothermia matched a decrease in ethanol minimum alveolar concentration (MAC), and thereby made the use of a single anesthetic concentration appropriate regardless of temperature. We determined ethanol MAC in normothermic (37.3°C) and hypothermic (28.5°C) rats, and, at the two temperatures, also determined ethanol solubilities in olive oil and saline. Ethanol MAC decreased, while olive oil/gas and saline/gas partition coefficients increased. However, the increase in the saline/gas partition coefficient did not match the decrease in MAC, and thus the aqueous-phase partial pressure producing absence of movement in 50% of rats (EC_{50}) values for ethanol decreased by 17%. Although this decrease is not large, it may be important for comparative estimates of the in vitro effects of ethanol at different temperatures.

Key words Anesthetics · Alcohols · Anesthetic potency—MAC · ED_{50} · Ethanol · Ethyl alcohol · Hypothermia · Meyer-Overton hypothesis

Although hypothermia decreases the partial pressures required to produce anesthesia (e.g., minimum alveolar concentration [MAC]) for potent, volatile inhaled anesthetics [1–7], room temperatures usually are used in studies of the effects of ethanol and other anesthetics in oocytes or isolated neurons. This change in potency mandates the use of different partial pressures at different temperatures to apply a constant anesthetic potency. However, Franks and Lieb [8] noted a counterbalancing effect; they observed that a decrease in temperature increased the solubility of potent inhaled anesthetics. Consequently, they suggested that a constant aqueous-phase concentration of anesthetic would produce an approximately constant anesthetic effect.

The suggestion by Franks and Lieb [8] of a constant anesthetic effect (MAC) at a constant concentration has been applied widely, particularly for potent inhaled anesthetics. However, their observations did not include ethanol, probably the most widely used anesthetic in the world (although it is not often thought of as an anesthetic). Ethanol may differ from potent inhaled anesthetics because of its greater polarity and affinity to water, and a potency far greater than its lipophilicity would predict [9]. For potent inhaled anesthetics, MAC awake (a measure of awareness) is approximately a third of MAC [10], but ethanol concentrations approaching MAC are required to suppress learning in a fear-potentiated startle paradigm in rats [11]. Although the temperature-dependence of the "anesthetic" effects of ethanol, and enflurane and halothane, has been studied in a crustacean (*Daphnia magna*) [12], the effect of temperature on ethanol MAC in mammals has not been studied. The differences between ethanol and potent inhaled anesthetics suggest that the effect of hypothermia on the MAC of ethanol might differ from its effect on potent inhaled anesthetics (i.e., might supply a more severe test of the above suggestion by Franks and Lieb.)

Because of an interest in the effects of alcohols, particularly ethanol, on receptors expressed in *Xenopus* oocytes, we tested whether, as found for potent inhaled anesthetics, a proportionate increase in solubility with hypothermia matched a decrease in ethanol MAC.

With approval of the Committee on Animal Research of the University of California, San Francisco, we studied 43 male specific-pathogen-free, Sprague-Dawley rats (Crl:CD(SD)BR), weighing 300–450 g, obtained from Charles River Laboratories (Hollister, CA, USA). The rats were divided into those maintained at

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normothermia (29 rats) and those subjected to hypothermia (14 rats; see below). Each animal was caged with up to as many as 2 additional rats, was exposed to a 12-h light-dark schedule, and had continuous access to standard rat chow and tap water before study.

To determine the hypothermic MAC of ethanol, for a given rat, we injected a total of 3.5 g·kg^{-1} to 4.5 g·kg^{-1} ethanol (20% dissolved in saline) intraperitoneally as two equal doses given 5 min apart. Temperature probes inserted rectally to a depth of 4–7 cm allowed continuous monitoring of temperature. Three minutes after the first injection of ethanol, we surrounded each rat with bags of ice. When the temperature reached 35°C, the rat was placed in an individual plastic cylinder, receiving oxygen at 1 l·min^{-1}. Ice placed under the cylinder continued the cooling to 31°C by 20 min after injection, at which time we removed the ice. At 30 min after the last (second) ethanol injection, temperature was 29.1 ± 0.7°C and at 40 min was 28.5 ± 0.1°C.

At 40 min, we applied an alligator clamp to the tail, moving the clamp up and down at approximately 1 Hz for up to 1 min, and observing the rat for movement of an extremity or the head. Forty minutes was used in our previous studies of n-alcohols [13], and this is the time of peak concentration in the rat brain after intraperitoneal injection of ethanol in normothermic animals [14]. If no movement occurred, we made an incision in the abdomen to permit withdrawal of approximately 10 ml of blood from the aorta into a heparinized 10-ml syringe. If movement occurred, we administered isoflurane (1%–2%) in 1 l·min^{-1} of oxygen to achieve anesthesia (lack of movement) and then obtained the blood sample. This sample was transferred to a 50-ml glass syringe.

We determined MAC for ethanol in a separate group of normothermic rats, using an identical approach, except that no ice was applied.

To determine the partial pressure of the ethanol in aortic blood, we added 20–25 ml of air to the syringe and equilibrated the contents for 60 min in an incubator at 28°C or 37°C. The gas phase was analyzed by gas chromatography for the ethanol (see below), using primary volumetric standards. Because of the great solubility of ethanol in blood, this gas-phase value immediately indicated the partial pressure in blood. We assumed that this partial pressure in blood was in equilibrium with the alveolar partial pressure (i.e., the alveolar concentration), and thus could be used in a determination of MAC.

We applied a logistic regression analysis to the resulting data [15]. Each rat supplied two values: the response (movement or no movement) and the associated alcohol partial pressure. The logistic regression analysis supplied a value for the partial pressure producing absence of movement in 50% of rats (the EC_{50}) and the variance (standard error) about this value. We defined this EC_{50} as MAC.

For solubility determinations, we determined the solubility of ethanol in saline and olive oil, using methods previously established in our laboratory [13].

For the gas chromatographic analysis, we used a Gow-Mac 580 flame ionization detector gas chromatograph (Gow-Mac Instrument, Bridgewater, NJ, USA). The 4.6-m-long, 0.22-cm internal diameter (ID) column was packed with carbowax. Column temperature was 103°C, and the detector was maintained at a temperature of 131°C. The carrier flow was nitrogen. The detector received 20–24 ml·min^{-1} hydrogen and 210–220 ml·min^{-1} air.

We found that ethanol MAC decreased by 44.3% with the imposed decrease in temperature. The saline/gas partition coefficient increased by 44.3% and the oil/gas partition coefficient increased by 29.4% (Table 1; Fig. 1).

Our finding of a 6.3% decrease (in gas-phase partial pressure) in MAC per 1°C decrease in body temperature approximates the change previously found with potent volatile anesthetics [1–7] and exceeds the change seen with cyclopropane [2] and nitrous oxide [4]. It assumes a proportionate decrease per degree temperature decrease. If a linear decrease occurs, then the decrease per degree is 5.0%.

Our results enlarge the suggestion of Franks and Lieb [8] to now include ethanol. At 37.3°C, the product of MAC times the saline/gas partition coefficient equals 314% atm and at 28.5°C it equals 253% atm. Thus, at 28.5°C, the concentration of ethanol is 0.1023 M, and at 37.3°C it is 0.1233 M. That is, at 28.5°C, the molar concentration is 17% smaller, indicating that hypothermia increases potency (defined either as a smaller concentration or partial pressure).

The 17% decrease may be an overestimate if the brain were at a lower temperature than suggested by our measurements. However, the temperature probe

Table 1. MAC values and partition coefficients

Temperature (°C; mean ± SE)	*n*	MAC (% atm; mean ± SE)	Olive oil/gas partition coefficient	Saline/gas partition coefficient	MAC times saline/gas part. coef.
37.3 ± 0.1	29	0.1160 ± 0.0022	118 ± 2	2710 ± 30	314% atm
28.5 ± 0.1	14	0.0646 ± 0.0033	153 ± 1	3910 ± 20	253% atm

MAC, minimum alveolar concentration of anesthetic required to eliminate movement in response to a noxious stimulus in 50% of subjects

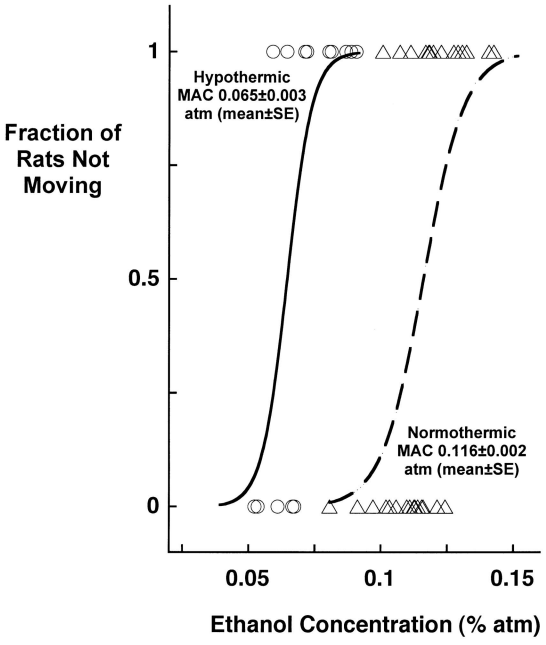

Fig. 1. We applied a logistic regression analysis to the data [15]. Each rat supplied two values: the response (movement or no movement) and the associated alcohol partial pressure in arterial blood (assumed to be in equilibrium with the partial pressure in alveolar gas). Each *open circle* indicates the data produced from a hypothermic rat; each *open triangle* indicates the data produced from a normothermic rat. The value for the partial pressure producing absence of movement in 50% of rats (the EC_{50}; i.e., minimum alveolar concentration [*MAC*]) and the variance (SE) about this value are indicated

was placed 5–7 cm into the abdomen, and the temperature changed only 0.6°C over the 10 min preceding clamp of the tail. It is unlikely that we overestimated the brain temperature of the rat by more than a fraction of a degree. A full degree overestimate would increase the saline/gas partition coefficient to 4070, giving a MAC times saline/gas partition coefficient value of 263% atm rather than 253% atm, or 0.1066 M rather than 0.1023 M, a 14% rather than 17% decrease from the 0.1233 M concentration at 37.3°C.

Our EC_{50} values for ethanol anesthesia (MAC) differ from those found by McKenzie et al. [12] in the crustacean *Daphnia magna*. Their EC_{50} values, determined at 25°C–30°C, were approximately 0.4 M, whereas we obtained a value of 0.1 M (Fig. 1). These different findings may result from different choices of experimental animals. Their much greater EC_{50} suggests that ethanol affects fundamentally different sites in the achievement of "anesthesia" in rodents versus crustacea. This notion also applies qualitatively to the potent inhaled anesthetics that they tested: McKenzie et al. [12] found that halothane and enflurane EC_{50} values exceeded those found in rats [16]. These discrepant potencies for ethanol and potent inhaled anesthetics may underestimate the actual differences, because the stimulus used to define "anesthetic" endpoints in McKenzie's study (failure to respond to a bright light) may provide a lesser stress than the application of a (supramaximal) tail clamp stimulus, as in our study. A large difference would strengthen the notion of a difference in the mechanistic basis for anesthetic effects in rodents versus crustacea. Another observation also suggests a different mechanistic basis for ethanol anesthesia in crustaceans versus mammals. The slope for the EC_{50} change with increasing temperature is negative for the crustacean and positive for the mammal (i.e., potency increases with increasing temperature in crustacea but decreases in mammals).

We add a philosophical, unanswerable, thought. The preceding comparisons of the results with rats versus the results with crustaceans assume that our measure of anesthesia in the rat (present study and Taheri et al. [16]) and McKenzie et al.'s [12] measure of anesthesia in the crustacean assess equivalent anesthetic states. Perhaps they do not and, if so, interpretations of the differences might be compromised.

Overall, our observations suggest a difference, albeit small, in the mechanisms underlying the actions of ethanol (and alcohol) and conventional inhaled anesthetics. However, differences in receptor effects are limited. For example, both alcohols and conventional inhaled anesthetics enhance the response of γ-aminobutyric acid $(GABA)_A$ and glycine receptors to their ligands [17]. Nonetheless, the prediction of potency from affinity to a lipid phase differs radically for alcohols versus conventional inhaled anesthetics [9].

In conclusion, our results suggest that the investigator who determines anesthetic potency at one temperature may not always assume that the amount of anesthetic dissolved at that temperature will produce an identical effect (will be equally potent) at another temperature. In the present study, the change in potency was modest. Whether it would always be so is not known, and may require determination.

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