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Temperature Dependence of the Potency of Volatile General Anesthetics

Implications for In Vitro *Experiments*

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Background: **When performing experiments at room temperature with volatile general anesthetics and *in vitro* mammalian preparations (such as isolated neurons), the question arises as to which concentrations of anesthetics are "clinically relevant." Different choices can lead to different interpretations of the anesthetic sensitivities of putative target sites.**

Methods: **Published data on the temperature dependence of minimum alveolar concentration were analyzed.**

Results: **Although gas-phase potencies of volatile anesthetics increase markedly with decreasing temperature, the corresponding aqueous-phase potencies are relatively constant. Changes in minimum alveolar concentration with temperature can be accounted for, on physical grounds, in terms of the temperature dependencies of anesthetics binding to their central nervous system target sites.**

Conclusions: **When performing room-temperature *in vitro* experiments on simple mammalian preparations with a volatile anesthetic, the aqueous-phase (but not the gas-phase) minimum alveolar concentration calculated at normal body temperature is, to a first approximation, the appropriate choice for a clinically relevant anesthetic concentration. Recommended aqueous-phase minimum alveolar concentration values (in mM) for desflurane, enflurane, halothane, isoflurane, and sevoflurane have been calculated. (Key words: Anesthetic potency. Minimum alveolar concentration. Temperature: hypothermia. Volatile anesthetics: desflurane; enflurane; halothane; isoflurane; sevoflurane.)**

THERE is an important practical dilemma facing those who perform *in vitro* laboratory investigations into the actions of volatile general anesthetics on mammalian preparations. This is the choice of the appropriate general anesthetic concentrations to use when the experimental preparation is at lower than normal body temperature. Different ways of interpreting anesthetic potencies in hypothermic animals can lead to very different ideas as to what constitutes "clinically relevant" concentrations at anything other than normothermia. In this article, we will discuss these different interpretations and offer some practical guidelines we hope will prove useful.

Volatile anesthetics are drugs that are (almost uniquely) administered clinically in the gas phase. Whereas these agents are, of course, applied to mammals at their normal body temperatures (usually about 37°C), for various technical reasons, experiments on simple molecular systems such as ion channels are often carried out at room temperature (20–25°C). Minimum alveolar concentration (MAC) invariably is determined using gas-phase concentrations (usually expressed as vol% = % atm). For example, mammalian MAC values for halothane are, on average,[1] about 0.9% atm. At first sight, it might seem reasonable to apply this same gas-phase concentration to an *in vitro* preparation at room temperature and assume that the effects observed are equivalent to those in the animal exposed to the same gaseous concentration. Is this procedure reasonable, or should some other method be used?

To answer such questions, it is necessary to consider quantitative animal data on how anesthetic requirements change with temperature. It is well known that, for a variety of volatile anesthetics, MAC (when expressed in % atm) can decrease markedly with decreasing temperature,[2–8] although the size of the effect varies from agent to agent. Because by far the most complete set of data is available for halothane acting on dogs,[2,3,5] we will use these results to illustrate some general principles that are characteristic of most volatile agents.

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These data (fig. 1, upper line) show that there is a roughly linear decrease in the halothane requirement as the temperature is decreased.

However, it could be argued that MAC values at anything other than normal body temperature cannot be interpreted easily because of the confounding variable of the physiologic effects of temperature. Obviously, mammals have complex mechanisms for maintaining normal body temperatures, and it is not unreasonable to suppose that a change in body temperature *per se* might influence anesthetic requirement. What is more, the decrease in the anesthetic requirement when linearly extrapolated predicts that one should require no anesthetic at all at a low enough temperature.[2] Remarkably, this does indeed seem to be the case. For example, thoracotomy on dogs has been found[9] to require no anesthetic at body temperatures between 15°C and 22°C, whereas in goats,[8] the MAC for isoflurane reduces to zero at 20°C. That a linear extrapolation of anesthetic concentration predicts this experimental finding seems to be, at first sight, a persuasive reason for believing that the effect of temperature *per se* is a progressive effect that is altering the anesthetic requirement at all temperatures other than the normal body temperature. We believe this logic is incorrect, for the following two reasons.

First, it has to be appreciated that, while the determination of MAC as a partial pressure is an excellent practical measure of anesthetic potency, choosing to *express* anesthetic concentrations in the gas phase is arbitrary. Provided the anesthetic is close to equilibrium,[10] the MAC concentration can equally validly be expressed as a concentration in gas or free aqueous solution. For example, in figure 1 (lower line) we have also plotted the halothane MAC data[2,3,5] as concentrations (mM) in water. When the data are looked at in this way, the anesthetic requirement changes very little with temperature (in the range 28–43°C), with the aqueous concentration varying only between 0.20–0.25 mM. (Using available potency[3,6] and partitioning[3,11] data, comparable changes, ~30% or less over a 15°C range, can be calculated for the volatile anesthetics isoflurane, methoxyflurane, ether, and fluroxene.) Not only is there relatively little change in anesthetic requirement, but the extrapolation predicts something quite different: a zero anesthetic requirement will not be reached until −40°C. Thus, the agreement between the gas-phase extrapolation and the point at which MAC drops to zero is completely fortuitous. (This also follows from the finding that gas-phase extrapolated temperatures can vary greatly from one anesthetic to another.[2]) Of course, at extreme temperatures (*e.g.*, 20°C in the goat[8]), there clearly are major physiologic changes that result in a vanishing anesthetic requirement. (Similarly, anesthetic EC_{50} values in goldfish tend to zero below about 2°C.[12]) For example,[13] canine cerebral metabolism decreases steeply below 27°C (the Q_{10} for the rate of oxygen consumption between 14–27°C is twice that for the range 27–37°C) and could be the cause of the steep decrease in anesthetic requirement at these extreme temperatures. However, although extreme temperatures may cause such gross physiologic changes in complex organisms (probably involving numerous molecular processes), it seems unlikely to us that such precipitous effects will be seen on, or are relevant to, the effects of general anesthetics on an individual molecule such as an ion channel studied at room temperature.

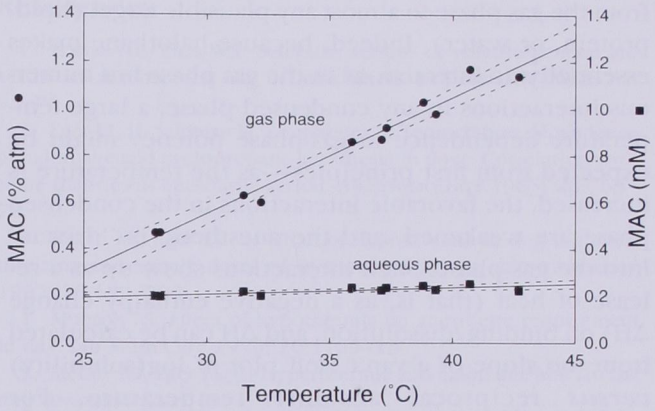

Fig. 1. Linear plot of halothane MAC *versus* temperature over the range 28–43°C. The data[2,3,5] are for dogs and are plotted both as gas-phase partial pressures (●; % atm) and as aqueous concentrations (■; mM). The latter were calculated[1] using Bunsen saline/gas partition coefficients, obtained for each temperature from a van't Hoff plot of the data in reference 11. The straight lines were fitted by the method of unweighted least squares, and the dashed lines represent the 95% confidence envelopes.

Second, one must consider that, to produce general anesthesia, volatile anesthetics must bind to, or dissolve in, their targets in the central nervous system, and this process itself will depend on temperature. This raises the question of the extent to which the temperature dependence of volatile anesthetic potency can be accounted for by simple solubility considerations.[2,8,14,15] For example, there is a decrease in solubility with increasing temperature when halothane is transferred

from the gas phase to almost any plausible target (lipid, protein, or water). Indeed, because halothane makes essentially no interactions in the gas phase but numerous interactions in any condensed phase, a large temperature dependence in gas-phase potency might be expected from first principles—as the temperature is increased, the favorable interactions in the condensed phase are weakened, and the anesthetic is "driven" into the gas phase. Such interactions show up as a release of heat (that is, as a negative enthalpy change ΔH) on binding/dissolution, and ΔH can be calculated from the slope of a van't Hoff plot of log(solubility) *versus* reciprocal absolute temperature. For halothane[11,16] dissolving from the gas phase into lipid bilayers or water, or binding to a protein (luciferase), ΔH is between about -35 to -60 kJ mol^{-1} (corresponding to a 2.0- to 3.3-fold increase in solubility/binding when the temperature is decreased from 37 to 22°C). This range, which presumably reflects the differing strengths of the interactions involved, encompasses the apparent $\Delta H = -52$ kJ mol^{-1} that has been calculated[14] from the observed[2,3,5] temperature dependence of halothane MAC for dogs. (The same value of ΔH also has been calculated for halothane anesthesia in goldfish[12]). To exert its effects, an anesthetic must bind to its primary targets, so there is, perhaps surprisingly, little if any temperature dependence left to be accounted for in terms of an additional effect of temperature *per se*. In other words, it appears that, provided the deviations from normal body temperature are not too great, the effects of temperature *per se* can, to a first approximation, be ignored.

This conclusion is consistent with the finding that the temperature dependence of anesthetic potency varies considerably among different volatile agents.[2–6,8] Further support for this view comes from the observation[15] that the rat gas-phase MAC for the simple gas nitrous oxide (which might be expected to interact relatively weakly with its targets[17]) decreases only minimally with decreasing temperature. However, the idea that the temperature dependence of animal potency can be accounted for largely by changes in solubility or binding needs to be tested experimentally by studying the effects of temperature on the interactions between volatile anesthetics and their most likely molecular targets, ligand-gated ion channels in nerve membranes.[18]

If gas-phase MAC values (fig. 1) change simply because of changes in solubility, then log(MAC) might be expected from elementary thermodynamics to change linearly with reciprocal absolute temperature. In fact, when the halothane data in figure 1 are replotted in this way (see van't Hoff plots in fig. 2), we see that the fit is equally good. Indeed, fits of the data from published[2–7] mammalian animal temperature studies to linear and van't Hoff straight-line plots cannot be distinguished (F test, $P > 0.5$; calculations not shown).

To avoid the complications and uncertainties caused by the inevitably large temperature dependences of gas-phase potencies, it is much simpler to consider a concentration in some condensed phase; water is as good as any other, but has the great practical advantage that anesthetics are often applied as aqueous solutions in *in vitro* experiments. (In experiments where the anesthetic can partition between biologic phases, it is, of course, the free aqueous concentration at equilibrium that is relevant.) When water is chosen, we see that aqueous halothane concentrations in the narrow range 0.18–0.23 mM should be used for canine preparations over the broad temperature range 20–37°C (see fig. 1). (The equivalent gas-phase concentrations can, of course, be used, but the large changes in gas-phase potency with temperature must then be taken into account.) Conversely, we see no justification for applying gas-phase concentrations measured at 37°C to experimental preparations at room temperature. In our view, this can amount to overdosing the preparation severalfold (see also reference 19). Many *in vitro* ex-

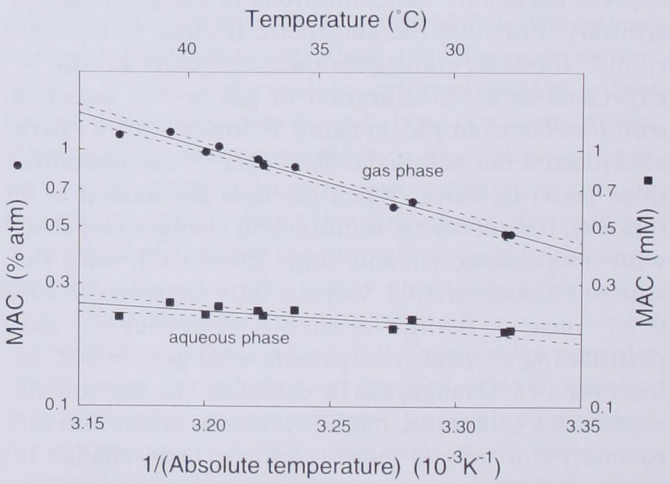

Fig. 2. van't Hoff plot of halothane MAC *versus* reciprocal absolute temperature. The data[2,3,5] are for dogs and are plotted on a logarithmic scale both as gas-phase partial pressures (●; % atm) and as aqueous concentrations (■; mM). The latter were calculated as described in the legend to figure 1. The straight lines were fitted by the method of unweighted least squares, and the dashed lines represent the 95% confidence envelopes.

Table 1. MAC Values Expressed as Partial Pressures in the Gas Phase and as Aqueous Concentrations (C_{aq}) in Saline

Agent	Animal	Partial Pressure (% atm)	C_{aq} (mM)
Halothane	Human[20]	0.75	0.19
	Rat[21]	1.03	0.27
	Dog[10]	0.87	0.23
Isoflurane	Human[20]	1.3	0.27
	Rat[21]	1.46	0.31
	Dog[10]	1.28	0.27
Enflurane	Human[20]	1.7	0.49
	Rat[21]	2.21	0.64
	Dog[22]	2.2	0.63
Desflurane	Human[23]	6.0	0.53
	Rat[7]	5.72	0.51
	Dog[24]	7.2	0.64
Sevoflurane	Human[25]	2.05	0.30
	Rat[26]	2.40	0.35
	Dog[27]	2.36	0.34

Values of C_{aq} were calculated[1] using the partial pressure values given in the table with literature values of Bunsen or Ostwald saline/gas partition coefficients at 37°C for halothane,[11] isoflurane,[11] enflurane,[20] desflurane,[28] and sevoflurane.[29] Although all data are for 37°C, the C_{aq} (but not the partial pressure) values are reasonable approximations for use at room temperature in *in vitro* experiments.

periments at "clinically relevant" concentrations actually are using free aqueous concentrations that would be close to lethal in animals at their normal body temperatures (the therapeutic indexes for volatile agents can be as low as 2–4).

It must be stressed, however, that all *in vitro* experiments on mammalian systems should, ideally, be carried out at normal body temperature. This will not always be either appropriate or convenient, however, and experiments will inevitably be carried out at other temperatures. In such cases, where reliable animal data at different temperatures exist, we would recommend that appropriate experimental anesthetic concentrations be obtained by extrapolating these data logarithmically (fig. 2) to the temperature of the *in vitro* experiment. However, if reliable animal data are not available, the best procedure would be to regard the free aqueous concentration achieved at 1 MAC in the animal at normal body temperature to be the appropriate benchmark.[1,18] For convenience, these values are given in table 1 for the volatile anesthetics most commonly used in current clinical practice.

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