Journal of Thermal Analysis, Vol. 50 (1997) 117-124

Dedicated to Prof. Menachem Steinberg on the occasion of his 65th birthday

A THERMOCHEMICAL ANALYSIS OF INHALATIONAL ANESTHETICS

Y. Katz[*] *and I. Aharon*[**]

[*]The National Physical Laboratory of Israel, Givat Ram, Jerusalem
[**]Department of Medical Biophysics and Nuclear Medicine, Hadassa University Hosphital, Jerusalem, Israel

Abstract

The mechanism of the anesthetic process is of interest both to the clinician and to the pharmacologist. However, this is still an unsettled issue and a multitude of models have been proposed for the process. Noticing that most models propose either a molecular perturbation by the agents or an effect on some colligative property, we explore in this article the thermodynamical consequences of these postulations. Comparison of these with experimental findings is then made. The comparison shows the inconsistency of many of the models with the facts: (i) it refutes the long accepted conviction, culminated in the 'unitary hypothesis', that general anesthetics act not at a particular receptor site but invariably on all. Some consequences of this finding are demonstrated. (ii) it implies that a simple phospholipid medium is not feasible as an anesthetic site. (iii) it infers that proteins do have the properties required from anesthetic sites.

Keywords: general anesthesia, mechanisms of anesthetic action, membrane bilayers

Introduction

General anesthetics is used for a complete and safe abolition of pain in surgical operations. A common practice involves the use of anesthetic gases and vapors, such as ethers, fluorocarbons and nitrous oxide. These agents act by depressing all excitable tissues in the central nervous system and the action is accomplished by interaction with neuronal membranes. The molecular mechanisms responsible to the loss of feeling in anesthesia is a central issue, still unsettled. However, since nerve membranes involve proteins and lipids, the question is usually presented in the form: does the primary site of action of anesthesia involve a protein, a membrane phospholipid environment or the boundary between both [1, 2]. A multitude of mechanisms concerning the action of anesthetics on these sites have been proposed most of which refer to the lipid bilayer (changes in membrane fluidity, effect on phase transition, changes in membrane thickness, are just some examples of suggested models). An inspection of these

0368–4466/97/ $ 5.00

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will show, however, that they all attribute anesthesia to either a molecular perturbation, a colligative property, or an adsorption process (the later is applied to proteins but not to the bilayer). We explore here the thermodynamics of the first two effects and show that they do not comply with the proposal that lipids are the primary sites of anesthetic action. The findings also put in doubt the applicability of the colligative mechanisms in general.

Molecular perturbations

Theoretical background

The relation connecting structural perturbations and the thermodynamic work of hole creation in the medium is an interesting one [3]. It is based on two observations:

(i) Structural changes are always expressible in statistical terms using pair correlation functions for the description. Perfect crystals can be described by pair correlation function which are δ functions with peaks at the lattice sites. The pair correlation function $g(r)$ of real liquids is deduced by X-ray diffraction and it reflects and characterizes the structure of these fluids. Peaks in $g(r)$ correspond to first neighbors, second neighbors, etc. These peaks become more and more smeared and their amplitude diminishes with the increase in distance between pairs until they finally fade away, reflecting the fact that liquids have only short range order. The representation of structural information in terms of pair correlation functions applies not only to crystals and liquids but also to those systems which are relevant to the investigation of anesthesia. Perturbations in structure that lead to anesthesia will, therefore, be manifested by changes in the pair correlation function of the medium.

(ii) Considering perturbations from a different point of view, we notice that the thermodynamical work of introducing an agent into the site can be described as resulting from two independent consecutive steps: creation of cavity in the medium and introduction of the solute into this cavity. The first of the two processes is relevant for the description of perturbations since the cavity is a space into which no part of the medium is allowed to enter whereas the space was filled with molecules of the medium prior to the creation of the cavity. Indeed the thermodynamical work W of creating a hole having the size of the solute can thus be expressed in terms of pair correlation functions (or by an equivalent description: the conditional probabilities that the centers of all solvent molecules are being excluded from a spherical shell, once the region enclosed by the shell is known to be free of molecular centers). Noticing the statistical nature of the representation is important for our work because it allows to apply the general and well known Boltzmann theorem of statistical mechanics. This theorem $P(r)=e^{-W(r)/kT}$ connects the reversible work $W(r)$ required to produce the cavity to the probability of forming this cavity by some thermal fluctuation. The force ex-

erted by the fluid on the surface of this sphere in the case of thermal fluctuation is the kinetic force due to encounters with the surrounding molecules of the medium. The basic equations showing the linkage between the various measures of perturbation are presented in detail in reference [3].

Anesthetic perturbations

Our knowledge of the mechanisms underlying general anesthesia are lacking as is our knowledge about the primary target site of anesthetic action. However, structural perturbations are generally assumed to be the cause of general anesthesia [1, 2], regardless of the considerable controversies concerning the molecular nature of the target site and the mechanism assumed. It has been recently shown that the thermodynamic work needed to generate such a perturbation is actually the work of creating a void (i.e. a cavity) in the medium that has the shape and size of the anesthetic agent [3].

The probability that such a cavity will be spontaneously formed by the thermal fluctuations of the site is not nil [3–4] and it is given by a Boltzman equation of the type

$$P = e^{-W/RT} \tag{1}$$

where P is a probability and W is the work needed to make a cavity. The equation implies that raising the temperature increases the probability of hole formation by a thermal fluctuation, meaning that heating, by itself, may (but not necessarily will) create anesthesia. More important, for the discussion that follows, is the implication that anesthesia can never be caused solely by low temperatures. A quick inspection shows that these conclusions are derived from first principles using only general arguments in the derivation.

Findings

This decisive conclusion that anesthesia vanishes by cooling, is contested, however, by the experimental findings. Cherkin's study, of anesthesia in goldfish, showed that at a sufficiently low temperature, anesthesia could be induced by cold alone, in the absence of anesthetics [6]. Similar results were obtained in a recent investigation, on goats, made by Antognini. He found, that hypothermia eliminates isoflurane requirements at 20°C [7].

The apparent paradox – a contradiction between physical first principles and experimental findings – can be resolved only by postulating two sites: (i) Perturbation by anesthetics occur at one site. This site has the ability to adsorb agents specifically and to be perturbed by their action. (ii) The second site is driven by thermal fluctuations and anesthesia commences when these diminish. Heating activates also the first site but the activation does not suffice to generate anesthe-

sia. The crux of this finding is in refuting the long accepted conviction, culminated in the 'unitary hypothesis', that general anesthetics act not on a particular receptor site but invariably on all. Some consequences of this finding are demonstrated below.

Consequences

(i) Let us first look into the implications for the lipid theories of anesthesia. Membrane lipids have only one site which can be perturbed. We and others have shown that a large number of anesthetic agents dissolve preferentially in the hydrophobic region of the bilayer [8–13], and perturb and medium (i.e. the hydrophobic region). The thermodynamic work needed to generate such a perturbation is the work of creating a void (i.e. a cavity) in the medium, described above [14]. Thus the hydrophobic region of the bilayer is not acceptable as a sole anesthetic site, because it offers only one site. Moreover, the likelihood of generating holes in it by thermal fluctuations increases with the increase in temperature whereas anesthesia is produced by diminishing the temperature. These facts rebut the view, held by most workers in the area, the solution process in bilayer lipids is the primary process of anesthesia [15].

(ii) In contrast to lipid bilayers, channel proteins are appropriate candidate target sites for anesthetic action, because they offer a possibility for several, temperature sensitive, modes of action. Channel action involve many kinetic steps, each step involves conformational changes of channel proteins and each change affects many parts of the macromolecule [16]. These traits are shared by all members of the broad family of voltage gated Na, K and Ca channels, which shared common kinetic and structural characteristics. (Ca channels have an additional unique role in that they translate electrical signals into chemical signals. They can thus regulate, by controlling the flow of Ca into the cytoplasm, a host of Ca dependent intracellular events [16–19] all of which may lead to anesthesia). The attribute of having many conformational changes involved in kinetic steps is shared also by other kinds of channel, such as the ligand-gated channels of fast chemical synapses [16]. It is interesting to mention in this connection the GABA-gated inhibitory channel whose response to drugs mimics to a certain extent the anesthetic effect: some drugs (for example picrotoxin and bicuculline) reduce GABA IPSPs (inhibitory post synaptic potentials) causing convulsions while others, known to have anesthetic effects (e.g. barbiturates, benzodiazepine tranquilizers and alcohols) potentiate the ISPSs and lead to calming and sedation [16].

(iii) The strong evidence that membrane lipids cannot be the sole anesthetic site while proteins can, reduces significantly the number of permissible lipid models. However, it does not imply that lipids cannot be involved in anesthetic processes. In fact no contradictions are found if the assumption is made that the lipid phase is the primary site of action of anesthetic agents, while anesthesia by

thermal action occurs at a different site. For example, it was found [20], that lowering the temperature of a squid giant synapse preparation (to 8.9°C±1.5 sdm) abolished activation of the postsynaptic action potential whereas the presynaptic spike was still generated. One might, therefore, assume, by analogy, that the action of anesthetic agents occurs at a postsynaptic site and may involve membrane lipids whereas temperature causes anesthesia by acting on a presynaptic site. An elimination of such a possibility would require the introduction of results by other experimental methods into the analysis. An example being the suggestion made [21] that observed potency changes for the inhalational anaesthetics cannot be accounted for in terms of changing solubility in lipid bilayers. Such arguments are, however, outside the realm of our discussion.

Colligative properties

Theoretical background

This discussion below involves two theoretical aspects: phase transition and solution behavior. The transition from one phase to another occurs at a fixed temperature which changes as the pressure or concentration change. This situation is usually represented graphically by a curve, which divides the phase diagram plane into two parts each of which describes the thermodynamical states of one phase. The equilibrium between a liquid solution and a pure solid solvent is thus described by the condition $\mu_1(T, p)=\mu_{1s}(T, p)$. The same applies to the pure solvent at its freezing point $\mu_1^\circ(T_o, p)=\mu_{1s}(T_o, p)$. Using these two relations, it has been shown [22] that $\Delta\mu_1(T)=\mu_1(T)-\mu_1^\circ(T)=\Delta_f H_1^\circ/R(1/T-1/T_o)$ where $\Delta_f H_1^\circ$ is the molar heat of fusion of the pure solvent. However, the chemical potential of the solvent is given also by the relation $\mu_1=\mu_1^\circ-RT_\varphi\Sigma m_s$ where φ is the osmotic coefficient and m_s are the concentrations of the solutes given in molar units. It thus follows that $RT_\varphi\Sigma m_s=\Delta_f H_1^\circ/R(1/T-1/T_o)$.

Phase transition and anesthesia

It has been proposed that phase transition of membrane phospholipids is the primary cause of anesthetic action [23, 24]. A similar mechanism was later suggested for anesthetic action on membrane proteins [25]. One model suggested that lateral phase separation the membrane followed by fluidity changes of the phospholipid matrix is essential for normal physiological function. Anesthesia interferes with this function by abolishing phase separation [23]. Another model [25] envisions nerve excitation as a transition between the two states of the excitation machinery consisting of proteins and lipids, proposing that both proteins and lipids change their conformation at excitation. It thus proposes that anesthesia occurs when compounds have a higher affinity to the resting state than to the excited state of excitable membranes, and that there is a critical tempera-

ture above which the affinity to the excited state becomes greater than to the resting state. When the temperature exceeds this critical level, compounds lose their anesthetic potency.

It should be noted when considering these models that they differ from the fluctuation – perturbation approach in their involvement with a constraint put on the perturbation rather than with the perturbation itself. The question of interest here is not in the connection between anesthesia and the perturbation produced by an introduction of an agent into the phase. Rather it is in the relation between two such perturbations, one in each phase, and the effect of the different agents on the balance between the two.

Findings

Supporting evidence

The evidence suggesting a phase transition mechanism of anesthesia were: (a) the response of models to pressure and anesthetic agents resembles the physiological response. For example, DSC measurements revealed that the gaseous anesthetics halothane and enflurane affect the transition temperature of multilamellar DPPC liposomes (they have no effect on the enthalpy of the transition). However the increase in pressure reverse the effect of anesthetics on both the transition temperature and transition width. This reversal of the effect parallels the phenomenon of pressure reversal by anesthesia [26]. (b) the existence of a cut-off phenomenon. Aliphatic solutes, paraffins, alcohols, esters and ethers depress the melting point of phospholipid membranes whereas the higher homologs of each series elevate it. This behavior parallels a similar cut-off effect in anesthesia.

Contrasting facts

Applying the equations derived previously to the later findings we gather that we can obtain a change of melting point in either direction by using a mixture of two solutes, one of which increases the melting temperature and another which decreases it. (The two agents will then produce a different osmotic coefficient for the 'anesthetized' medium).

Since the effect of solutes on phase transition temperature is of two kinds it is expected that the introduction of long aliphatic molecules would counteract the effect of the short chain solutes. Such an effect has not been demonstrated yet in anesthesia. Rather, an additivity of potency has been observed for nearly all anesthetic mixtures studied [27]. For example, it was found that a molecule such as *n*-decane is not anesthetic despite having an adequate liquid solubility and no known anesthetical effect which may interfere with its anesthetic action [28]. Furthermore, the additivity of action of anesthetic agents is strongly related to other very basic findings culminated in the Meyer-Overton hypothesis. This hypothesis suggests that narcosis is induced when the concentration level of the in-

ert gas dissolved in the lipid phase exceeds a specific threshold value. A consequence of this assumption is that additivity of the effect of two gases on the lipid can and are to be assumed. However, the hypothesis that phase transition is the cause for anesthesia has to be rejected since it predicts the abolishment of anesthesia by the larger solute molecules if additivity of action of agents persists.

Remarks and conclusions

1) First finding: Perturbation is a possible cause of anesthesia and the only possible cause if membrane phospholipids are the site of anesthetic action. It can be shown, by inspection of the Boltzmann equation that a sufficiently large thermal fluctuation will also cause anesthesia in such systems. But, anesthesia can never be produced solely by low temperature, if there is only one kind of anesthetic site in the medium. The conclusion follows that since membrane phospholipids have only one possible relevant site they are not a possible anesthetic site.

2) Second finding: Colligativity and additivity of action of anesthetic agents contradict each other. A mechanism in which an anesthetic agent influences the temperature of phase transition is a mechanism in which the colligative properties of the site are affected. If different molecules have different effects on the site (while their concentrations are the same) then this means that the osmotic coefficient of the medium is affected differently by these molecules. This demand follows from the thermodynamics of the colligative properties. However, it is known that anesthetic agents act additivity on the site (consistent with the Meyer-Overton rule). Molecules with sizes larger than that of the cut-off agent described in the mechanism of Ueda can either act additively (algebraically) or colligatively but not in both mechanisms at the same time.

3) The arguments of paragraphs 1) and 2) when applied to the mechanisms existing for membrane phospholipids as the site of anesthetic action eliminate most of them.

4) Double site mediums, consistent with the demands presented here, are of two kinds: (i) the two sites are on the same molecule. This applies to proteins (where two sites of different action have been found). (ii) the two sites may reside on two different molecules or even on different cells.

5) Arguments 3) and 4) imply that a simple phospholipid medium is not feasible as an anesthetic site. They also infer that proteins do have the properties required from anesthetic sites.

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