Mechanisms of anaesthesia: a mystery

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The search for the mechanisms of anaesthesia still continues in the midst of an era of molecular neurobiology. It has been stated¹ that "the mystery of anaesthesia, like that of consciousness, has not yielded to molecularity." How and where anaesthetics act remain a mystery; a mystery that has intrigued and frustrated many for several decades.

Anaesthesia is a complex phenomenon. It is not a simple state that can be easily defined. Described as a reversible loss of sensation, awareness and consciousness,² the unique spectrum of effects produced by each anaesthetic does not allow for a precise definition. A further complication is created due to a lack of a clear definition of the state of consciousness or understanding of how the brain functions.

To lend some clues to the mystery, considerable progress has been made in describing the physical characteristics and nature of the site(s) of action.^{3,4} This subject has been well documented in a number of recent reviews and monographs.^{3,5-9}

A unified mechanism of action

Early theories of anaesthesia focused on a common or unified mechanism of action.⁵ Since a large number of simple molecules of diverse chemical structure are capable of producing anaesthesia, a specific site or receptor appeared to be not involved. The lack of a structural requirement for activity is unique to neuropharmacology; one reason for the difficulty in determining the mechanisms. All anaesthetics share a common property of relative high lipid solubility (often defined as an oil/water partition coefficient). This physicochemical parameter appears to be one of the major factors related to activity.

The historical account of Meyer and Overton^{4,5} at the turn of the century marked the beginning of the universal non-specific (physical) mechanism of anaesthesia later termed the lipid solubility hypothesis. The original reports demonstrated that anaesthetic potency was correlated with solubility in an oil phase. K.H. Meyer¹⁰ later formulated the theory of anaesthesia which stated that anaesthesia "commences when any chemically indifferent substance has attained a certain molar concentration in the lipids of the cell." Often referred to as the Meyer-Overton rule of anaesthesia (see reference 4), this statement identified a hydrophobic region as a site for anaesthetic interaction. The correlation provides a means of predicting relative anaesthetic potencies, and thus is a "rule" of

efficacy; but does not provide any data for a theory of mechanism.⁷ It has, however, been utilized by many investigators to support a common mechanism of action for general anaesthetics. The "rule" was modified in later years attempting to provide an explanation for the mechanism, referred to as the "critical volume hypothesis."¹¹ The "critical volume hypothesis" which states that anaesthesia occurs when the volume of a hydrophobic region is caused to expand beyond a certain critical volume as a result of adsorption of an anaesthetic into the membrane phase, was consistent with observations that anaesthetics could expand membranes, bilayers, monolayers, bulk phase solvents, oils and even rubber⁷ and that pressure could reverse anaesthesia.

Studies using spin probe and fluorescence spectroscopy demonstrated that anaesthetics increase membrane fluidity; that is, most anaesthetics caused a transition of lipids from the ordered (gel) phase to the disordered or fluid (crystalline) phase. These studies supported the critical volume hypothesis and membrane expansion theories (see reference 7). The changes in membrane dimension or fluidity in the presence of surgical levels of anaesthetics may be relatively minor when compared to effects that occur with small changes in temperature.⁶ It was argued, therefore, that if small changes in temperature could not produce anaesthesia, then the alterations (expansion or fluidity) in membrane properties have little to do with anaesthesia.

There is still a fundamental controversy whether anaesthetics act directly on the hydrophobic regions of proteins or indirectly via the lipids that surround the proteins. Which of the membrane components is the principal target is not resolved, although current opinions favour direct action with proteins.⁶

Sites of anaesthesia

A molecular mechanism of action must explain the effects of anaesthetics at the level of the neuron and the whole organism. Although the precise site within the central nervous system is unknown, synaptic transmission has been regarded as the primary cellular site of anaes-

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thesia.¹² However, the effects at non-synaptic regions or subregions of synaptic membranes must be considered in the overall effect.⁷

Anaesthetics directly alter membrane properties, neurotransmitter efficacy, receptors, chemical and voltage-gated ion channels and enzymes (see references 3, 7, 12). The function of each neuron is dependent on the coupling of these factors, therefore focusing on a single site could result in the description of a mechanism that is secondary to anaesthesia.⁴ It is highly probable that several sites and/or mechanisms are involved, many of which overlap.

It is still controversial whether general anaesthesia results from depression of excitatory transmission, facilitation of inhibitory synapses, a decrease of spontaneous firing or a direct suppression of neuronal excitability. There is considerable evidence from neurophysiological studies that all anaesthetics do not produce a similar state of anaesthesia.^{2,7,13,14} Each agent can produce a very characteristic pattern of effects and the pharmacological profiles include both depression and activation. A common (non-specific) mechanism of action does not provide an explanation for the spectrum of effects observed in the whole animal.¹³ A mechanism of simple depression does not explain how the major portion of the central nervous system can continue to function normally during anaesthesia, or how each agent produces a unique state of anaesthesia. We must be cautious not to unify the actions of all anaesthetics with a similar mechanism such as non-specific perturbation,⁵ hyperpolarization¹⁵ or Ca^{2+} -dependent K^{+} conductance.²

Selective sites of action

It has been well documented that the actions of anaesthetics are selective at the level of the whole animal,¹³ synapse¹² and membrane.^{14,16} Highly specific actions, for example, presynaptic reduction of transmitter release or selective enhancement of inhibitory transmission also occur.^{2,19}

The idea of a common mechanism has dominated the literature for over a century but is now being challenged. Specific actions suggests selective "sites" which are capable of discriminating structural differences: therefore, interactions can be influenced by molecular size, shape and charge of the agent, in addition to hydrophobicity. This has been shown for barbiturates,^{14,17} steroid anaesthetics,¹⁸⁻²⁰ halogenated volatile anaesthetics²¹ and long-chain alcohols,²² and is in agreement with a multi-site hypothesis.²³

Biological activity of anaesthetics is usually associated with simple molecules of low molecular weight suggesting that the sites are restrictive in both size and physical property. The potencies of a homologous series of anaesthetic agents progressively increase with molecular

weight (or size) until a limit is reached suddenly at which the anaesthetic potency disappears. This phenomenon is termed the "cut-off" effect and can also be explained on the basis of selective sites of action. There are still major disputes as to the precise point of cutoff and the interpretation of results.^{24,25}

Differential and selective effects of anaesthetics *in vitro*

During the past few years, we have demonstrated the selective effect of a variety of anaesthetic agents using two *in vitro* preparations. The *in vitro* studies were not directed at the phenomenon of anaesthesia *per se*, but provide data which ultimately may explain the mechanisms involved in the state of anaesthesia. *In vitro* preparations provide the advantages of controlled environments, known concentrations, precise location of recording and stimulating electrodes and quantitative measurements of neuronal and synaptic activity.

Our recent studies have demonstrated, using the crayfish sensory neuron and rat hippocampal brain slice preparation as model systems, that general anaesthetics produce differential and selective actions that are structure, concentration- and time-dependent.^{7,14,16,17,21,26} A variety of anaesthetic agents produced biphasic responses on the discharge activity of the sensory neuron.²⁶ Although a correlation was found to exist between effective concentrations and lipid solubility (partition coefficient), it was not possible to provide an explanation for the differential actions. We proposed selective interactions with "sites" which could discriminate structural differences of the molecules. Three prototype anaesthetics (halothane, alcohol and pentobarbital) were also found to produce different concentration-dependent membrane effects suggesting multiple sites and/or mechanisms of action.¹⁶ The effects of barbiturates on the two models also revealed selective and pathway specific effects.¹⁴ In addition, a comparison of the stereoisomers of pentobarbital demonstrated the ability of neuronal membranes to discriminate subtle structural differences.¹⁷ Volatile anaesthetics applied as vapours in clinical concentrations to the models exhibited differential effects; enflurane and ether induced seizure-like bursts in both preparations^{21,27} as recorded *in vivo*. Isoflurane, a structural isomer of enflurane, however, only produced a depression of activity.²¹

Anaesthetic recognition sites

The results of these studies support the concept that general anaesthesia is a complex phenomenon involving multiple mechanisms and sites, and not a simple depression of neuronal activity. It is suggested that anaesthetics interact with "recognition sites" that are differentially localized within neuronal membranes. The term "recogni-

tion sites" is not to be associated with classical receptors, and future investigations may resolve the identity and characteristics of these sites. In addition to the physico-chemical properties and molecular structure of the anaesthetic, the composition of the membrane must be considered in the differential effects of the anaesthetics.²⁸ It still remains to be determined what membrane components or subunits are directly involved in the interaction. A greater understanding of the molecular and cellular events of neuronal function is required before the pharmacological actions of each anaesthetic can be interpreted. Perhaps continued probing into the "Black Box" will provide a solution to the mystery of anaesthesia.

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