STUDIES ON A PROPOSED COMMON MECHANISM FOR THE ACTION OF GENERAL AND LOCAL ANAESTHETICS IN THE CENTRAL NERVOUS SYSTEM

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ABSTRACT

Procaine and several general anaesthetics have been reported to block action potential production in frog's skeletal muscle fibres and nerve fibres by a single mechanism of action suggesting a common basic mechanism of action on all excitable cells. It was proposed, therefore, that these agents produce their effects on the central nervous system by a similar common mechanism.

Procaine, dibucaine, lidocaine, cocaine and a convulsant barbiturate 5-ethyl-5-(1,3-dimethylbutyl) barbiturate (DMB) given alone to intact white mice produced 'excitement' and convulsions but when given 60 minutes after phenobarbital caused central nervous system depression. Large convulsant doses of these agents caused a loss of the righting reflex in mice pretreated with small subanaesthetic doses of phenobarbital. In contrast, pentylenetetrazol only antagonized the depression produced by phenobarbital. Pentylenetetrazol given after a combination of phenobarbital and procaine antagonized only the phenobarbital depression and added to the depression produced by procaine.

When applied topically to neuronally isolated slabs of cat's cerebral cortex, procaine or pentobarbital reduced the sizes of the surface negative response and surface positive burst response to direct stimulation to the cortex. Pentylenetetrazol had the opposite

effect. DMB usually depressed both responses of the cortex.

When given systemically, procaine and high doses of DMB raised the threshold for the surface positive burst response, ether raised or did not change this threshold and pentylenetetrazol and low doses of DMB either lowered the threshold or left it unchanged. In addition, DMB in low doses lowered the threshold for the surface negative response.

Systemically administered procaine and pentylenetetrazol usually increased the primary component of the click evoked response in the auditory cortex of spinalized cats. In contrast, the amplitude of this response was decreased by subanaesthetic doses of thiopental. Pentylenetetrazol given after thiopental increased the amplitude of the evoked response over that produced by thiopental alone. Procaine, depending on the dose, potentiated or antagonized the thiopental response.

The results support the contention that local and general anaesthetics act by a single common basic mechanism in the central nervous system. It is suggested that the differences in the central nervous system effects observed after local and general anaesthetic administration is due to the greater degree of conduction block produced by local anaesthetics on small fibres in inhibitory pathways.

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I. INTRODUCTION

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A. GENERAL INTRODUCTION

In 1846, Morton demonstrated the general anaesthetic properties of ether and in 1884, Koller introduced cocaine as a local anaesthetic into clinical practice (Goodman and Gilman, 1955a). Many new drugs having these effects have been introduced since that time and various theories have been proposed to explain these effects on nervous tissue. Since both general and local anaesthetics act on excitable tissue, theories concerning the mode of action of one group might apply to the other as well. The present investigation is based on the concept that both groups of drugs have a common basic mechanism of action on nervous tissue at the cellular level.

B. CHEMISTRY

Those agents usually classified as general anaesthetics have a wide divergency in chemical structure. Until 1939, these agents included little more than various analogues of short chain hydrocarbons and a wide variety of urea derivatives which included the barbiturates. The discovery of the anaesthetic effects of argon (Behnke and Yarborough, 1939) added a completely new type of anaesthetic compound. Later, krypton and xenon were also shown to be anaesthetics (Lawrence et al., 1946). It becomes clear, then, that no specific structure is absolutely necessary for anaesthetic activity, and indeed, this lack of specificity can be regarded as an outstanding characteristic of anaesthetics (Butler, 1950).

Despite this lack of structural specificity there are nevertheless some structural features which appear to be incompatible with anaesthetic properties. These features have been summarized by Butler (1950), and are presented here. First, active anaesthetics have not been found among organic compounds that are ionized to a large extent at physiological hydrogen ion concentrations. Compounds that are highly soluble in water are also poor anaesthetics. The introduction of some group e.g., hydroxyl, which increases the water solubility of a compound by the formation of hydrogen bonds, reduces anaesthetic activity. Finally, metabolic reactions leading to strongly ionized or highly soluble products are important in terminating the effects of many anaesthetics. This is particularly true for the barbituric acid derivatives (Burger, 1960a). Predictions on the lack of anaesthetic activity based on structure alone can be made with considerably more confidence than can predictions of the presence of such activity (Butler, 1950).

In contrast to this lack of structural specificity in the general anaesthetics, the clinically used local anaesthetics constitute a relatively homogeneous chemical group. For the most part they are tertiary amino esters of aromatic acids although the ether and amino analogues may also show local anaesthetic activity. In addition to the para-amino benzoic acid derivatives, quinine and quinoline derivatives are also active, as are several primary and secondary aromatic alcohols. The structural features of the local anaesthetics have been reviewed by Hirschfelder and Bieter (1932).

All antihistaminic drugs are also capable of exerting a limited degree of local anaesthesia if applied topically. These agents are all chemically related to each other but bear only a very remote resemblance to the clinically used local anaesthetics. There appears to be no correlation between local anaesthetic effectiveness and antihistaminic activity (Goodman and Gilman, 1955b). A major difference between these two classes of drugs is their effects on the central nervous system. Systemic administration of the clinically used local anaesthetics will, in adequate doses, produce central convulsions. The antihistaminics given in this way, may produce sedation or convulsions (Goodman and Gilman, 1955b).

C. COMPARISON OF THE EFFECTS OF GENERAL AND LOCAL ANAESTHETICS AT THE CELLULAR LEVEL

The classification of general and local anaesthetics into separate categories may be artificial. Gros (1929) showed that cocaine, eucaine and procaine prevented the normal motion of paramecia. Conversely, cutaneous injections of chloroform, ethylurethane and several other general anaesthetics into the centre of a wheal in mammals, produced local anaesthesia. Alcohol and ether have been shown to produce conduction block when applied directly to peripheral nerve (Davis et al., 1925). Cocaine and amyl alcohol were shown to produce peripheral nerve block by stabilization of the cell membrane i.e., block without depolarization (Bishop, 1932) and similar results have been obtained for the barbiturates (Heinbecker and Bartley, 1940). Low concentrations of homologous series of urethanes were found to produce a small hyperpolarization of the cell membrane while depolarization occurred with higher concentrations (Cresticelli, 1948). Conduction block in the nerve, however, occurred both during depolarization and hyperpolarization.

Any similarity between the effects of ether and those of local anaesthetics has been obscured by observations indicating that ether prevents the action potential in nerve and muscle by depolarization of the membrane rather than by stabilization (Wright, 1947; Lorente de No, 1947; Heinbecker and Bartley, 1940; Alcock, 1906). The evidence, however, is equivocal. Gross and Cullen (1943), for example, observed that ether reduces the response of skeletal muscle to nerve stimulation and to close intra-arterial injection of acetylcholine. They also showed that ether enhances the effect of curare (which stabilizes the membrane) and could be antagonized by neostigmine, a cholinesterase inhibitor which blocks impulse transmission by depolarization.

Strong evidence for the stabilizing action of ether has been obtained recently by Yamaguchi (1961) and by Inoue and Frank (1962a). It was shown that ether blocks the production of action potentials in skeletal muscle fibres by inhibiting the specific increase in sodium conductivity of the membrane which normally follows an adequate stimulus. Procaine, a local anaesthetic which has consistently been shown to block by stabilization of the membrane (Bennet and Chinburg, 1946; Bishop, 1932), also blocks action potential production by an identical mechanism in skeletal muscle (Inoue and Frank, 1962b) and in nerve (Taylor, 1959; Shanes, et al., 1959).

There exists, therefore, a distinct possibility that both local and general anaesthetics act by some single basic mechanism of action at the cellular level. The concept is not new, but between the first unified field theory of Claude Bernard (1875) and the microcrystal hypothesis of Pauling (1961), many theories of narcosis (or anaesthesia) have been advanced.

D. HISTORICAL REVIEW

1. Theories of general anaesthesia and their relevance to local anaesthesia

One of the controversies which has plagued theories of general anaesthesia has been the use of the terms 'narcosis' and 'anaesthesia'. The multiplicity of meanings given to the former have made this term almost meaningless unless specifically defined. As used in discussion of the following theories of anaesthesia, narcosis will be taken to mean the general depressant effect produced by drugs. As such, this term will also include anaesthesia and may be substituted for it. The reverse substitution i.e., anaesthesia for narcosis, may not be made. Anaesthesia, as used below, will be considered to be a special case of narcosis implying a loss of consciousness in some animal, or, if used in the clinical sense, includes Stage I (analgesia), Stage II (excitement), Stage III (surgical anaesthesia).

None of the theories discussed below explains adequately the mechanism by which various agents produce anaesthesia. The purpose in reviewing these theories is to point out how unsatisfactory is our knowledge about anaesthesia and to show in what respects these theories failed to elucidate anaesthetic mechanisms.

a) The Overton-Meyer Theory

H.H. Meyer (1899) proposed a theory of narcosis which was almost immediately endorsed by Overton (1901). It is based on the fact that narcotics are soluble in lipoids and that the strength of their action is related to their distribution coefficient in oil and water. Meyer stated his theory in the following way:

1) All chemically indifferent substances which are fat solvents exert a narcotic action upon living protoplasm insofar as they can diffuse into it.

2) The effect shows itself first and most strongly in those cells where there is a preponderance of lipoid material viz., the nerve cells.

3) The relative efficiency of such narcotics is dependent on their physical affinity for lipoids on the one hand and for water on the other hand. It is dependent therefore, on the partition coefficient which determines their distribution in water and lipoid.

Both Meyer and Overton used an olive oil-water system in testing this hypothesis although they were aware that neutral fats probably did not exist as a normal component of the cell membrane. On the basis of the proposed theory, the depressant effect on the cell would parallel the molar concentration of narcotic in the cell lipoid independent of the chemical structure of the narcotic. K.H. Meyer and Gottlieb-Billroth (1920), however, tested a large number of anaesthetics and found that the narcotic concentrations, calculated by multiplying the concentration of the substance necessary to produce narcosis by the olive oil-water partition coefficient, varied between .001 for ethylurethane to .09 for phenylurethane - a factor of almost 100. Much better agreement was obtained for the volatile anaesthetics, the range being between .04 for ethylene and .10 for amylene (Meyer and Hopff, 1923).

Thus, from the outset, the Overton-Meyer theory could not account adequately for all anaesthetics. Meyer and Hemmi (1935) suggested that oleyl alcohol be substituted for olive oil as it appeared to give a much closer correlation with narcotic effectiveness in tadpoles than did the olive oil-water system. Lofgren (1948), however, decided that local and general anaesthetics could not be compared even with this improved model, since substances with widely differing activities appeared to have the same partition coefficients. He concluded that the minimum effective concentrations of local anaesthetics were not a function of the partition coefficients alone, but showed nevertheless

that these agents were most effective in the lipid phase i.e., local anaesthesia was greater in oil than in water.

The most serious blow to the Overton-Meyer theory was given by Winterstein (1926). He showed that narcotics are capable of exerting their effects on organisms which are completely free of lipoids e.g., acetone-extracted yeasts. It is obvious, therefore, that the Overton-Meyer theory of narcosis is incorrect, since a fat layer of some type is required to give the hypothesis meaning.

From this and other observations, Butler (1950) suggested that the oil-water partition coefficient is merely a measure of the barrier through which the drug must pass in order to exert its effect. This interpretation permits an explanation of the observation that two drugs with the same partition coefficient may produce effects ranging from depression to convulsions as occurs, for example, with several barbiturates (Albert, 1960a).

b) The Traube Hypothesis

A second physico-chemical theory of narcosis was advanced by Traube (1904). He observed that many narcotics were included among a large group of substances which lowered interfacial tension between water and some other phase. He suggested that a definite relationship existed between the surface activity and the narcotic strength of a drug. According to the Gibbs adsorption equation (Glasstone, 1946), the lowering of the surface tension of a solution by a substance is directly proportional to the degree that it accumulates at the surface. Traube regarded this theorem from a different point of view. He stated that the more a substance lowered the surface tension of its solution, the less it attached to the main body of the fluid and this could be

measured by changes in the capillary activity at an air-water interface. The implications of this theory are that narcosis is achieved when the interfacial tension at the cell membrane is lowered to a critical point which is independent of the chemical structure of the surface-active agent. Later, Traube (1912) suggested that since alkalisation increases the surface activity of alkaloids, the increased activity of local anaesthetics in alkaline solution could be attributed to this cause.

The adsorption theory is fraught with many difficulties. Ethylene, ethyl chloride, chloroform and carbon tetrachloride do not lower the interfacial tension between oil and water (Lazarew, 1930). Soaps and detergents, which lower the interfacial tension of an oleyl alcohol-water model do not possess narcotic properties (Meyer and Hemmi, 1935). Among these compounds are naphthalene sulphonates, cetyl sulphonate and salts of fatty acids with carbon chains of six units or more. The Traube theory is also incompatible with the observation that both lidocaine and chloroform increased the surface tension of a cholesterol film (Lofgren, 1948). More recently, Luduena and his co-workers (1955) tested 37 local anaesthetics for surface tension lowering ability. No correlation was obtained for local anaesthetic potency and surface activity although a positive correlation between irritancy and surface activity was found. Finally, Traube's experimental model can be criticized. Values obtained for capillary activity at an air-water interface are merely a measure of wettability and probably bear no resemblance to the events occurring on cellular surfaces (Hober, 1945; Albert, 1960b).

c) The Warburg Theory

Verworn (1912), suggested that narcotics act by interfering with cell oxidations, i.e., anaesthesia could be considered as a type of asphyxia. Some presumptive experimental evidence supporting this hypothesis was supplied by Warburg (1914) using a charcoal catalyst. He showed that narcotics inhibit the oxidation of amino acids by occupying the catalytic surface. He later showed that with larger narcotic molecules, fewer of them were required to coat a given surface area of the charcoal and to produce a given degree of catalyst inactivation. He claimed, therefore, that the anaesthetic activity of a drug is totally dependent on the degree to which it is adsorbed onto the cell surface (Warburg, 1921). In support of this theory, Lofgren (1948) suggested that in vivo the anaesthetic adheres strongly to the node surface of the nerve fibres which results in the formation of a highly concentrated layer of the drug. When this concentration reaches a critical value, anaesthetic effects result. Hober (1945), however, criticized this hypothesis on two counts. First, he pointed out that some narcotic substances are not adsorbable on any cell surface and secondly, some substances which are strongly adsorbed exert no anaesthetic effect.

Watson (1960) considers that adsorption can potentially influence two processes. It might decrease metabolism by blanketing the oxidative process or it might affect permeability by decreasing porosity. Both of these points are covered by other theories and it is unlikely that adsorption alone can explain anaesthesia.

d) Ferguson's Hypothesis

Ferguson (1939) and later Brink and Posternak (1948) attempted to resolve the discrepancies between the foregoing theories. They suggested that chemical potential is a more suitable index of anaesthetic activity than is concentration in oil or adsorption on charcoal surfaces. This concept is based on the assumption that all substances can exert a physiological effect by a physical process. If an equilibrium exists between the external phase and the biologically affected phase, the chemical potential must be the same in each phase. The thermodynamic potential in the external phase can be measured and therefore its value in the biologic phase is known. For gaseous substances, the activity is given by the ratio of the partial pressure of the gas mixture (p_t) required to produce anaesthesia, to the saturated vapour pressure of the substance (p_s) at the temperature of the experiment. If the anaesthetic agent is in solution and is of limited solubility, the activity of the anaesthetic concentration can be put approximately equal to S_t/S_o, where S_t is the molar concentration of the narcotic solution and S_o its solubility in moles per litre. Ferguson applied these calculations to a wide variety of volatile anaesthetics and showed that there was a narrow range of values into which many of these substances fell. He concluded that the principal cause of narcosis was the same for all drugs giving the same p_t/p_s values. He assumed that these agents had a non-specific action which was purely physical in nature. Those agents whose p_t/p_s values fell outside this range were assumed to be acting both by a physical and a chemical mechanism. Ferguson's calculations imply that the ability of the drug to reach the biological phase is more important than whether the agent is soluble in oil or whether it is

adsorbed at the surface of the cell. It seems likely, however, that the specific chemical action of the drug at the biologically affected phase is also of considerable importance.

There is no doubt that Ferguson's calculations are based on sound thermodynamic principles. A major criticism of this work must be that there is no evidence to support the contention that all substances can exert a physiological effect by a physical mechanism. No cognizance is taken of the possibility that when an agent reaches the biophase its physiological effect may result from chemical union with some enzyme system preventing it from carrying out its normal function. A further weakness in this work is that the calculations do not apply to all anaesthetics and two separate and distinct theories are required - one to explain the physical effects and one the chemical.

e) Metabolic Theories

All the metabolic theories are based on the premise that anaesthetics interfere with cell oxidations resulting in a type of asphyxia. The first proponent of such a theory was Verworn (1909). He suggested, on the basis of some rather meagre evidence, that during anaesthesia the ability of a cell to absorb oxygen was impaired. At about the same time, Heaton (1910) showed that a stimulated nerve became inexcitable before a non-stimulated one when both were placed in a narcotic solution. Verworn explained this by assuming that utilization of oxygen is greater when the cell works regardless of whether or not it is narcotized. He further postulated that the ability of the cell to take up oxygen is lost in the presence of anaesthetics. According to this theory, an increase in the duration of narcosis would cause a greater asphyxia.

Over the years, much experimental evidence has appeared to support this theory of anaesthesia. Mansfeld (1909) showed that polywogs are anaesthetized by lower concentrations of paraldehyde in the absence of oxygen and Hamburger (1912) observed that anaesthetics prevented the cell lipids from absorbing oxygen. Kisch (1913), although unable to confirm Hamburger's work, nevertheless held that many anaesthetics decrease the absorption of oxygen by cells. Green and Curry (1925) and Brown et al., (1927) presented evidence that nitrous oxide is a better anaesthetic at low oxygen concentrations but they felt that a considerable part of the anaesthesia was due to anoxia. There is little doubt that the earlier experiments can also be interpreted in this way.

More conclusive evidence can be cited to show the inadequacy of Verworn's hypothesis. Warburg (1910) observed that fission of sea urchin eggs was inhibited by urethane and phenylurethane but that oxidation was little affected. Much higher concentrations of various urethane derivatives were found to be necessary to inhibit oxidation of frog brain slices than to produce anaesthesia (Usui, 1912). Winterstein (1915) observed that the same concentration of ethanol required to produce anaesthesia in frogs resulted in an increased oxidation in nervous tissue. He also demonstrated that frogs anaesthetized with urethane, a long-acting anaesthetic, could be revived within two minutes by perfusion with oxygen-free saline. Pigeons anaesthetized continuously for 14 days, recovered with no ill effects (Ellis, 1923). Certainly the high metabolic rate in birds should be affected by anoxia within a short period of time. Finally, Warburg and Wiesel (1912) showed that the anaerobic growth of brewer's yeast could be halted by several urethanes and alcohols and obligate anaerobic bacteria were also shown to be capable of being