ON THE MODE OF ACTION OF LOCAL ANESTHETICS[1]

By J. M. Ritchie and Paul Greengard

Albert Einstein College of Medicine, New York, N.Y. 10461, and Department of Biochemistry, Geigy Research Laboratories, Ardsley, N.Y. 10502

Local anesthetics possess the specific ability to block conduction in nerve in a reversible manner and in low concentration. The reactions that occur in the nerve membrane that allow local anesthetics to achieve this effect are still not clearly defined. However, with the recent increase in our knowledge of the nature of the nerve impulse, some idea of the mode of action of local anesthetics is beginning to form. Measurements of membrane potentials and ionic movements have strongly suggested that local anesthetics block conduction by interfering with the sodium and potassium conductance changes that are fundamental to the generation of the nerve impulse. Other studies have indicated that local anesthetic molecules achieve this conduction block in the form of cations. Furthermore, studies of structure-activity relations have suggested something of the chemical nature of the receptor in the nerve membrane and the method of interaction of this receptor with the local anesthetic molecule. Finally, studies of the behavior of local anesthetics in model systems have suggested a physicochemical basis for the mode of action of local anesthetics on the nerve membrane. These various aspects of local anesthetic action will be given particular emphasis in this review. Many other, perhaps equally important, aspects will be neglected, and the interested reader is therefore referred to the many comprehensive reviews on local anesthetics that have appeared in the last decade (4, 14, 24, 25, 56, 73, 94, 104, 106, 108).

Electrophysiological Effects of Local Anesthetics

The work of Hodgkin, Huxley, Keynes, and others [see (52)] has led in recent years to a better understanding of the nature of the nerve impulse. In consequence, it is now possible to explain the action of local anesthetics within the framework of the ionic theory of nervous activity.

Active nerve membrane.—Local anesthetics increase the threshold for electrical excitation in nerve, slow propagation of the impulse, reduce the rate of rise of the action potential, and eventually block conduction [e.g. (85)]. They do this by interfering with the process fundamental to the generation of the nerve action potential, namely the large transient rise in the permeability of the membrane to sodium ions that is produced by a slight depolarization of the membrane. Taylor (99), Shanes et al. (85), and, more recently, Blaustein & Goldman (19) used the voltage-clamp method to study

[1] The survey of the literature pertaining to this review was concluded in October 1965.

the effect of procaine and cocaine on the electrical properties of the membrane of the squid and lobster giant axons. They concluded that the blocking action of local anesthetics is the result of a simple reduction in the carrying capacity of the system that allows sodium ions to be transported across the membrane in response to concentration and electrical potential gradients. The fact that local anesthetics interfere with the sodium carrying system is further emphasized by the experiments of Condouris (26, 27) who found that the conduction block caused by cocaine can be alleviated simply by raising the external sodium concentration. Furthermore, the effect of sodium deprivation on the conduction of the nerve impulse is qualitatively similar to that produced by cocaine (26, 66).

The local anesthetics also reduce the increase in potassium conductance that occurs in response to a depolarizing step in voltage, but the effect is much smaller than that on sodium conductance (85, 99). This reduction of the potassium conductance would tend to lower the threshold and thus partly counteract the blocking action of local anesthetics.

Resting nerve membrane.—Local anesthetics also reduce the permeability of resting nerve to sodium and potassium ions. However, higher concentrations are required than those needed to affect the permeability changes produced by depolarizing voltage steps (85). This accounts for the observation that, in the concentrations required to produce block in conduction, there is not any large or consistent change in the resting potential (17, 18). Straub (98) has shown that the small changes in membrane potential that do occur depend on the initial physiological state of the nerve fibers; thus, procaine produces a slight rise in potential in a normal nerve, a slight fall in a nerve in a catelectrotonic state, and no change at all in a nerve in an anelectrotonic state.

From a study of the effect of procaine, at various values of pH, on the relation between resting potential and external sodium and potassium concentration, Straub (97) concluded that the uncharged form of procaine causes the decrease in resting sodium conductance, and the charged form the decrease in resting potassium conductance. Unfortunately, there are no comparable studies in the literature on the transient conductance changes, which are the important changes as far as block is concerned. For example, in the voltage-clamp studies no information was obtained on how the action of procaine is affected by the external pH.

Muscle membrane.—A reduction in permeability to sodium and potassium ions also occurs in the membrane of muscle, both in the resting state and during the generation of an action potential. Shanes (80) has shown that the swelling of muscles in solutions of high potassium content, a property dependent on the entry of potassium chloride, is inhibited by cocaine. Inoue & Frank (55) concluded, from a study of the current/voltage relations in frog skeletal muscle fibers impaled with intracellular microelectrodes, that procaine suppresses the specific increase in the sodium conductance that generates the action potential. The increase in potassium conductance with

depolarization is also reduced by local anesthetics, but the chloride conductance is unaffected (3). Both anomalous and delayed rectification are abolished by cocaine (41).

As in nerve, local anesthetics decrease the rate of rise of the muscle action potential, reduce the overshoot, and delay the fall of the spike (36, 57). In concentrations lower than those required to produce block, therefore, the muscle action potential is prolonged, a phenomenon which might account for the increase in muscle twitch tension produced by local anesthetics (36).

Frog skin.—Frog skin is able to transport sodium from the outside of the skin to the inside; and the sodium transport can be measured by the current that is necessary to "short-circuit" the potential across the skin (58, 105). Skou & Zerahn (95) used this "short-circuit" current as a measure of sodium ion movements and concluded from experiments in which local anesthetics were applied to the outer surface of the frog skin that the uncharged form of the local anesthetics causes a decrease, and the cationic form an increase, in the permeability of the outer surface of the skin to sodium ions. Thus, at pH 6.0 where practically all the local anesthetic was in the form of the cation, they found an increase in permeability to sodium, whereas at pH 10.0 where nearly all the anesthetic was present as the uncharged base, they found a decrease in the permeability to sodium. Addition of N-methyl procaine, the quarternary analogue of procaine, to the solution bathing the outer surface increased the sodium permeability at every value of pH.

Biochemical Aspects of Local Anesthetic Action

Several naturally occurring substances, such as acetylcholine, ATP, calcium ions, and thiamine, have each been postulated to play a key role in the conduction of the nerve impulse. Recently there have been a number of reports of interaction of local anesthetics with the above agents. The possibility exists therefore that local anesthetic action is mediated through an interference with the natural function of one or other of these substances. It must be emphasized, however, that any such interference would have to be at the nerve membrane, there being conclusive evidence that local anesthetics act here. Furthermore, the local anesthetics do not act indirectly by depressing the general metabolism of the cell. Thus, the character of the block that can be produced by known metabolic inhibitors, such as 2, 4-dinitrophenol, is quite different from that produced by local anesthetics; block produced by metabolic inhibitors is slow and is accompanied by a depolarization, whereas block produced by local anesthetics develops rapidly and with little or no change in membrane potential. Finally, it should be noted that any theory on the mode of action of local anesthetics based on demonstrated antagonisms between local anesthetics and a naturally occurring substance is weakened by the very diversity of structures that are able to antagonize local anesthetic activity.

Acetylcholine.—For many years the interesting possibility has been considered by Nachmansohn and his colleagues that acetylcholine has a critical role to play in the conduction of the nerve impulse (64, 65). Therefore, the fact that interactions have been observed between local anesthetics and acetylcholine is of great interest. Local anesthetics repolarize electroplax cells that have been depolarized by acetylcholine and carbamylcholine (51), or prevent the depolarization from occurring in the first place(15). Furthermore, dibucaine reduces the size of the acetylcholine-induced depolarization of mammalian C fibers by roughly the same proportion as it reduces the size of the compound C potential (71). Conversely, acetylcholine can arrest, or even reverse, the depression in the action potential produced by procaine in frog single nerve fibers (20).

Such results seem to support the hypothesis, put forward in 1943 by Thimann (101) on the basis of the structural similarity of procaine to acetylcholine, that local anesthetics act by competing with acetylcholine at some membrane site that is involved in the conduction of the nerve impulse. However, there is considerable controversy over a physiological role for acetylcholine in nervous conduction (70). Furthermore, although acetylcholine does indeed resemble procaine structurally, the resemblance of acetylcholine to a number of other potent local anesthetics, such as phenacaine, is less obvious.

It seems unlikely that the block of conduction by local anesthetics is mediated through an inhibition of acetylcholinesterase. Thus, although the local anesthetics do inhibit both pseudo (6, 91) and true (92) acetylcholinesterase, there is no correlation between the relative potencies of the compounds as inhibitors of the enzyme and their ability to block impulse conduction. Nor is there any similarity between the pH dependency of the inhibition of acetylcholinesterase and the pH dependency of conduction block (91, 92).

ATP.—Kuperman et al. (60) have made the interesting observation that ATP can prevent, and even reverse, the block in conduction produced by procaine on isolated frog sciatic nerve; as little as 1 m*M* ATP virtually completely antagonizes the action of 5 m*M* procaine. This action of ATP, which requires the presence of calcium ions in the external solution, is shared by ADP and AMP but not by related compounds such as adenosine, creatine phosphate, or ribose-5-phosphate. The mechanism of the interaction of ATP with local anesthetics is not clear. That ATP is not acting by complexing with procaine is indicated by the fact that it is effective in the presence of a fivefold excess of anesthetic. Nor does it seem likely that ATP antagonizes procaine by enhancing the passive influx of sodium ions through a direct action on the transport mechanisms that are affected by local anesthetics. For example, ATP does not affect the size of an action potential that has been reduced by bathing the preparation in a sodium-deficient medium (60).

Although the adenine nucleotides do antagonize the ability of local an-

esthetics to block conduction, in other respects the nucleotides behave similarly to the local anesthetics. For example, ATP, ADP, and AMP (but not adenosine, creatine phosphate, or ribose-5-phosphate) antagonize the spontaneous activity that occurs in calcium-deficient nerves (67). A similar antagonistic effect on the spontaneous activity of calcium-deficient nerves is obtained with procaine (67). This latter effect is reminiscent of the effect of procaine in antagonizing the depolarization of frog nerve that occurs in calcium-deficient solutions (28, 97). The two effects of procaine presumably reflect the same, primary, stabilizing action.

There is at least one important difference between the stabilizing effect of the adenine nucleotides and that of local anesthetics. Calcium is not necessary for the stabilizing effect of procaine, but the presence of some free extracellular calcium ions is essential for the maximum stabilizing effect of the nucleotides (67). Okamoto, Askari & Kuperman (67) concluded from the great speed with which the stabilizing action of the nucleotides occurs, that they act at the plasma membrane, presumably by forming a lipoprotein-calcium-nucleotide complex (1).

These results and conclusions are of particular interest because of the role that ATP plays in metabolism. However, too little is known at present to speculate on the part, if any, that ATP plays in the process of local anesthesia. The facts given above, that ATP and procaine are each capable of stabilizing calcium-deficient nerve, whereas ATP antagonizes the block brought about by procaine, emphasize the complexity of the situation.

Calcium.—Both calcium ions and local anesthetics "stabilize" excitable membranes. For example, in the presence of either, the electrical threshold is raised, spontaneous discharges are reduced in frequency or abolished, and conduction of impulses may be blocked with practically no change in resting potential (21, 39, 81, 82, 85). It has long been suspected that calcium has a critical role to play in the generation of the nerve impulse; thus, it has been suggested that it is the removal of calcium ions from sites or carriers in the nerve membrane by depolarization that leads to the transient increase in sodium permeability during the action potential (21, 40, 47, 82). The hypothesis has been put forward [e.g. (107)] that calcium ions and local anesthetics act on the same system, namely the system that is responsible for carrying sodium ions through the nerve membrane. This would explain, for example, why the depolarization of bundles of desheathed frog myelinated fibers produced by calcium-free solutions can be completely reversed by procaine (28, 97). Feinstein [(37); see, however (38)] has made the more specific suggestion that the primary action of a local anesthetic is to inhibit the release of calcium from the sites to which it is bound in the membranes. As a result, the changes in sodium and potassium permeability that follow calcium release do not occur, and generation of the impulse is prevented.

The view that calcium and local anesthetics have the same site of action is strongly supported by the recent voltage-clamp experiments of Blaustein

& Goldman (19) who studied the competitive interaction of calcium and procaine on the sodium conductance of lobster giant axons. They showed that an increase in the external calcium concentration reduces the effectiveness of procaine, and that a decrease in the external calcium concentration increases it. They concluded that calcium and procaine compete with one another with respect to their actions on the membrane conductance mechanism. It is of great interest in this connection, therefore, that procaine and its analogues compete with calcium for binding to phospholipids *in vitro* (38), particularly since it has been proposed (45) that the polar heads of membrane phospholipids may serve as a gating mechanism that controls the transient changes in membrane permeability during the action potential.

In some respects the actions of calcium and local anesthetics seem to be opposed. Thus, whereas local anesthetics depress the increases in permeability of the nerve membrane to sodium and potassium ions that occur during the nerve impulse, calcium has the opposite effect, from which it was concluded that calcium and local anesthetics have different sites and mechanisms of action (85). A similar conclusion was reached from studies of the intracellularly recorded electrical activity of neurons of isolated frog spinal ganglia (2). However, this conclusion does not seem necessary in terms of current receptor theory. Both the agonistic and the antagonistic effects of calcium and procaine would seem to be readily accounted for on the assumption that procaine competes with calcium for the same receptor sites that control the sodium and potassium conductances, but that procaine is less effective because of differences in the kinetics of the reactions of the two substances with the receptor.

Thiamine and other substances.—A variety of other naturally occurring substances have been claimed to modify, usually antagonistically, the action of local anesthetics [see (108)]. These include certain water-soluble vitamins, particularly thiamine; the amino acids, aspartic acid, tryptophan, leucine, and cysteine; the veratrum alkaloids; and urea. A clear-cut involvement of thiamine in local anesthesia would be particularly interesting in view of the role proposed for this vitamin in the conduction of the nerve impulse (63). The reports of the interaction of thiamine with local anesthetics have, however, been conflicting. For example, it has been reported (50) that thiamine antagonizes the action of procaine in topical, infiltration, and nerve block anesthesia; on the other hand, the same report claims that thiamine prolongs the duration of procaine-induced spinal anesthesia.

Charge-transfer complexes.—Recently Eckert (30, 31) has reported that a large number of local anesthetics form donor-acceptor complexes (π electron complexes) with thiamine, the local anesthetic acting as the electron donor and the vitamin as the acceptor. Eckert postulated that the formation of such complexes in living nerve fibers might be responsible for local anesthesia. There are, however, several objections to this interesting suggestion. First, thiamine, if involved in nerve conduction at all, is most likely to be involved in the form of the coenzyme, thiamine pyrophosphate; and it

remains to be shown that the coenzyme form can serve as an acceptor in a charge-transfer complex with local anesthetics. Secondly, it is by no means certain that the local anesthetics would form charge-transfer complexes *in vivo* with thiamine, either in the free or coenzyme form. Thirdly, although the fascinating work of von Muralt and others [see (63)] on a possible relation between thiamine and impulse conduction is provocative, an essential relation remains to be proven.

Agin (5) has concluded that the charge-transfer bands observed by Eckert are artifacts, produced by his method of spectrophotometric analysis. Publication of further experimental data might help to resolve this conflict.

Agin (5) has reported the formation of a "charge-transfer complex" between procaine and ribonucleic acid. According to this report, the essential nucleic acid component appeared to be the ribose, and it was suggested that cell surface mucopolysaccharides may play an important part in the action of certain anesthetics. However, since ribose does not contain a π electron system it is not clear how it would participate in a π electron complex. Nevertheless, the experiments of Eckert and of Agin emphasize the possibility that local anesthetics may owe their blocking effects to their ability to form charge-transfer complexes with constituents of the nerve membrane.

The Active Form of Local Anesthetics

It seems clear that the molecular basis of the generation of the nerve impulse and the molecular basis of the action of local anesthetics are but two facets of the same problem, and that a better understanding of either would almost certainly lead to an improvement in our knowledge of the other. It is largely for this reason that considerable attention has been devoted to determining the active structure of local anesthetics. Most local anesthetics of clinical usefulness are secondary or tertiary amines. These compounds may exist both as uncharged molecules (B) and as positively charged substituted ammonium cations (BH^+), the relative proportions depending on the pH of the solution and on the pK_a of the anesthetic according to the relation,

$$pH = pK_a - \log (BH^+/B) \qquad 1.$$

Much effort has been devoted to the question of whether it is the uncharged or the charged form of the molecule that reacts with receptors in the nerve membrane to produce anesthesia.

The argument for the uncharged molecule.—Trevan & Boock (103) found that less anesthetic was required to block conduction when applied in an alkaline than in an acidic or neutral medium, from which they concluded that the uncharged molecule is the active form. This observation has been confirmed by many investigators with the same conclusion (32, 43, 78, 86). However, there is not close agreement between the observed minimum effective concentration at different values of pH (Fig. 1, curve *C*), and that

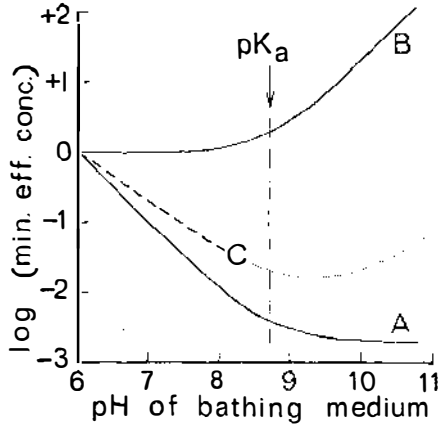


FIG. 1. The effect of pH on the minimal effective concentration of a local anesthetic. The concentration at pH 6.0 is taken as unity. For detailed explanation, see text.

calculated on the assumption that the uncharged molecule is active (Fig. 1, curve *A*). In the experiments of Skou (86), for example, the amount of anesthetic required to produce anesthesia fell by only one half the amount theoretically expected when the pH of the bathing medium was increased by one pH unit. However, Shanes (81), examining Skou's data on blocking potency as a function of pH, has rightly pointed out that the theoretical calculation should have been based on activities rather than on simple concentrations. The theoretical and observed curves for cocaine are then said to become identical (81). Unfortunately, the calculations supporting this statement are not documented, although it was indicated [(81), p. 139] that the theoretical calculation is based on Skou's (87) data on the variation of solubility of this anesthetic with pH. This statement on the correspondence between the theoretical and observed values has been widely accepted [e.g. (14, 94)]; it seems worthwhile, therefore, to examine its basis.

According to Skou (88), the pK_a of cocaine is 8.7 at 22° C. If the activity coefficients of the base and the cation are assumed to be equal (and they are commonly both taken to be unity) about 1/6 of the total anesthetic would be present in the uncharged form at pH 8.0 and only 1/51 at pH 7.0. Thus, if equal concentrations of base were required to produce the same degree of anesthesia at the two different values of pH, then 51/6, i.e., 8.5 times as much total anesthetic would be required at pH 7.0 as at pH 8.0. This factor is much larger than that determined experimentally by Skou [(86), Table 2], who found that only about four times as much anesthetic was required. A more reasonable calculation would be to determine, as Shanes (81) suggested, the relative amount of total anesthetic at the different values of pH that would produce a constant activity of uncharged molecules in solution. This calculation can be made for highly concentrated

solutions of anesthetics. For in a series of saturated solutions of local anesthetics at different values of pH, the activity of the uncharged base in solution must remain constant because it is the same as the activity of the undissolved anesthetic. We have calculated from the water solubility curves of Skou [(87), Fig. 1], as Shanes (81) presumably did for cocaine, the relative amounts of local anesthetic required at different values of pH to produce a constant activity of uncharged base in solution. In order to proceed further in the absence of other data, it is necessary to make the questionable assumption Shanes (81) presumably made, that this relation also holds for the more dilute solutions of the local anesthetics required to produce anesthesia. We have examined in this manner each of the five local anesthetics used by Skou. On this basis, six times as much cocaine would be required at pH 7.0 as at 8.0 to produce equal activities of uncharged base in solution. Thus, in the case of cocaine only part of the discrepancy between the original theoretical value of 8.5 times, based on concentration, and the experimentally observed value of about 4 times, can be accounted for. Moreover, cocaine is the only one of the five local anesthetics studied by Skou (86) for which it is possible to reduce the discrepancy by means of this type of calculation. Similar calculations for tetracaine and dibucaine increase the discrepancy between the theoretical and observed values. No such calculations can be made for the remaining two anesthetics studied by Skou (tropacocaine and procaine) because there is no overlap in the solubility and blocking potency data. In summary, therefore, the use of activities estimated in this way does not reconcile the experimental results with the predictions of the theory that it is the uncharged molecule that is active. In any case, as indicated above, there is considerable doubt about the validity of such a calculation.

A more reasonable approach is that of Löfgren (61) and Ehrenberg (32) who assumed that the activity coefficient of the uncharged molecule is constant, and is equal to unity, whereas that of the charged cation (f_{BH^+} may vary. The corresponding relation to Equation 1 then becomes

$$pH = pK_a - \log (BH^+/B) - \log f_{BH^+} \qquad 2.$$

An analysis on this basis has indeed been carried out by Ehrenberg (32) but only for one local anesthetic (4-methyl lidocaine) and, unfortunately, over a small range in pH (only 6.96 to 7.39), over which range f_{BH^+} was taken to be constant. Equation 2 could conceivably account for the discrepancy between the theoretical and observed values of the minimum effective concentration at different values of pH if f_{BH^+} were to decrease progressively as the value of the pH at which the experiment is carried out is increased. However, as the experimental pH increases, less anesthetic is required, and the ionic strength of the solution decreases; it is extremely unlikely that the activity coefficient of the local anesthetic cation would decrease with decreasing ionic strength.

For these reasons, the statement that the minimum effective concentra-

tion of a given anesthetic is such as to give a constant activity of free uncharged base in the bathing medium must be regarded as unproven. In any case, the mere demonstration that a constant amount of uncharged base is required in the bathing medium would not prove that the uncharged molecule is active; it might, as Ehrenberg (32) pointed out, just result from the anesthetic acting on the other side of a barrier that is permeable only to the uncharged form. This will be discussed more fully below.

The argument for the cation.—At the time when it was still generally believed that the active form of a local anesthetic was the uncharged molecule, evidence in favor of the cationic form began to accumulate. Thus, in 1940, Krahl, Keltch & Clowes (59) made a careful study of the action of local anesthetics in inhibiting cell division and concluded that the presence intracellularly of the cationic form was responsible for the inhibition. Largely on the basis of these experiments, Goodman & Gilman (46) suggested, in 1955, that the form of the molecule that is active in blocking impulse conduction in nerve fibers is the cation. In 1959, Ehrenpreis (33, 34) isolated a protein from the electric organ of the electric eel, which he believed to be involved in conduction. This protein was found to react strongly with the cationic but not with the uncharged form of local anesthetics (35). Bartels et al. (16) argued that if local anesthetics blocked electrical activity in conducting tissues by combining with some active site similar to this protein, then the cationic form of a local anesthetic should have a stronger action than the uncharged molecule on the electrical activity of the isolated electroplax. By studying the effect of pH on the minimum concentration required to produce block, they showed that this was indeed the case. However, the electroplax is a tissue in which acetylcholine has a well defined physiological role. It can be argued, therefore, that the action of a substituted ammonium cation at this site is merely a reflection of its interaction with the acetylcholine system here, and may have nothing to do with the blocking action of local anesthetics on nerve, where the role of acetylcholine in conduction is controversial [e.g. (64, 70)].

A major difficulty in obtaining an unequivocal answer to the question of the active form in which local anesthetics block conduction in nerve axons has resulted from the fact that the effectiveness of a local anesthetic is determined by two factors (*a*) the penetration from the site of application to the site of action, and (*b*) the actual anesthetic action at the receptor site. Ritchie & Greengard (72) attempted to minimize the effect of penetration by using desheathed nerve preparations. They found, in contrast to the earlier findings, that local anesthetics were more effective in neutral than in alkaline solutions. This finding, which was confirmed by Dettbarn in experiments on single myelinated fibers of the frog (29), provided strong evidence that the blocking activity of a local anesthetic resides in the cationic rather than the neutral form of the drug. The evidence that led Ritchie & Greengard (72) to conclude that the cation is the active form came from experiments with nerves that had been pretreated with dibucaine, a local

anesthetic with long-lasting effects, until complete block occurred; all subsequent observations were made in anesthetic-free solutions. Through the use of this procedure, the factors involved in the penetration of the local anesthetic did not need to be considered when interpreting the results. Following removal of the local anesthetic from the surrounding medium, the nerves remained blocked for as long as the pH was maintained near 7.2. But, when the pH of the solution bathing the nerve was raised to 9.6, conduction was dramatically restored; on being returned to pH 7.2 conduction was again blocked. Thereafter, conduction could be repeatedly restored and blocked just by switching between pH 9.6 and pH 7.2. Since the local anesthetic had been withdrawn from the bathing medium, it seems clear that the greater degree of block in the more acidic solutions was caused by the increased proportion of the residual anesthetic within the membrane in the active, i.e., cationic, form.

Another implication of these experiments is that binding to the receptor site and anesthetic action are two, somewhat separable, processes. A general explanation for the separation of these two functions may be that the local anesthetic molecule is bound to the membrane by its lipophilic, aromatic portion, and that its hydrophilic, amino group, acting in the cationic form, is responsible for the anesthetic action. The presence of the fixed local anesthetic cation in the membrane might lead to an electrostatic repulsion of sodium and potassium ions attempting to cross the membrane, with a consequent decrease in sodium and potassium conductances.

Further support for the cation being the active form has recently been provided by experiments in which the rate of block was studied at different pH values (74). Tertiary amines penetrate tissues (22, 49, 54, 59, 68), including nervous tissue (74), more readily than do the corresponding substituted ammonium ions. If the uncharged molecule were active, therefore, both the rate of penetration of the anesthetic through the periaxonal tissue, and its effectiveness at the site of action, should be greater in alkaline than in neutral solution. For these two reasons, the rate of block would necessarily be greater in alkaline solution. However, the measurement of the rate of block in desheathed preparations shows that the cationic form of an anesthetic is much more effective than the uncharged form, in spite of the reduced ability of the cation to penetrate any tissue barriers (see below) left after removal of the epineurium.

It might have been possible that the effects of pH on the action of local anesthetics were caused by a change in the reactivity of the nerve membrane rather than by a change in the molecular form of the local anesthetic. However, experiments in which the electrical threshold and the size of the action potential (48, 72, 74) were determined, and experiments with drugs whose molecular form is largely unaffected by pH, such as butanol and carbachol (74, 86), showed that any effects of pH on the nerve membrane are slight or absent in the range of pH values used in the studies of the nitrogenous local anesthetics.

The interpretation of the dependence of blocking potency on pH.—The conclusion drawn from the experiments just described was that local anesthetics penetrate nervous tissue as uncharged molecules, but react in their cationic form with the receptors in the nerve membrane to produce anesthesia (*72, 74, 75*). However, a different view has long been maintained, namely that the uncharged molecule is the active form in producing conduction block. This view was based essentially on experiments such as those of Trevan & Boock (103) and of Skou (86), which indicated that the minimum effective concentration necessary for producing local anesthesia decreases as the pH is increased. However, as Ariëns & Simonis (*7*, 8) have pointed out, this latter conclusion is based on a faulty analysis, which results from neglecting to take into account the difference between the pH of the solution bathing the nerve and that in the immediate vicinity of the receptors. A sample calculation illustrates this point. Consider the case of a nerve whose sheath is intact and which is exposed to a given concentration of a local anesthetic whose pK_a is 8.7. When the pH of the medium is increased from 7.7 to 9.7 there will be a tenfold increase in the amount of uncharged molecule in the bathing medium. If, as is generally agreed, the uncharged molecule is free to move across the tissue barriers, the concentration of uncharged molecule in the immediate vicinity of the receptor will increase tenfold. If, therefore, the base is active in producing anesthesia, only one tenth as much anesthestic will be required in the external medium. However, if the tissue buffers maintain the pH in the region of the receptors at a constant value, the amount of cation here must also increase tenfold. In fact, if the pH in the vicinity of the receptors remains constant with variation of external pH, the amount of cation must always change in parallel with that of the uncharged molecule. The mere experimental observation, therefore, that less anesthetic is required in more alkaline solutions does not permit any conclusion concerning the active form [see also (32)].

Thus, if the pH in the region of the receptors remains constant, the same relation between minimum effective concentration and pH will be obtained whichever form is active. The slope of this relation (Fig. 1, curve *A*) will be minus one at all pH values greatly below the pK_a of the anesthetic and zero at all pH values greatly above the pK_a. It is unlikely that the pH in the region of the receptors will remain constant. However, whatever happens to the pH in this region, the concentration of uncharged molecules in the vicinity of the receptors will always be equal, at equilibrium, to the concentration of the uncharged form in the external medium. Thus, if the uncharged molecule were active, curve *A* would always be obtained. If, however, the cation were the active form, the relation between minimum effective concentration and external pH would depend on what happens to the pH in the immediate vicinity of the receptor. Thus, if the pH in the periaxonal space exactly follows the pH in the external medium, curve *B* would be obtained. The slope of curve *B* is practically zero at all pH values greatly below the pK_a and is equal to plus one at all pH

values greatly above the pK_a. However, if the pH in the periaxonal fluid only partially follows the pH in the bathing medium, as seems most likely, an intermediate curve, similar to curve *C*, would be obtained. The exact shape of this curve would depend on the relation between the pH in the bulk of the solution and that in the periaxonal space. The slope of such intermediate curves lies between zero and minus one at all pH values greatly below the pK_a. The portion of curve *C* between pH 6.0 and 8.0 was actually drawn from Skou's results for cocaine. At pH values greatly in excess of the pK_a, the slope lies between zero and plus one (dotted portion of curve *C*), but this latter point has never been tested experimentally.

Thus, if the uncharged molecule were active, curve *A* (slope minus one) would always be obtained regardless of the pH in the vicinity of the receptors. However, if the cation were active, curve *A*, *B*, or an intermediate curve *C* would be obtained according to whether the pH in the receptor region remains constant, follows exactly, or follows only partially the pH in the external medium, respectively. The fact that no author has obtained curve *A*, whereas many authors (43, 78, 86, 103) have obtained curve *C*, argues strongly against the uncharged form and in favor of the charged cation as being the active form of local anesthetics. Trevan & Boock (103) attempted to reconcile their data (curve *C*) with the hypothesis that the base is active by postulating the presence of a pH barrier between the external medium and the membrane. However, as explained above, whatever happens to the pH in the vicinity of the membrane, curve *A* should always be obtained if the uncharged base is the active form.

The influence of the epineurium.—In all studies in which the external nerve sheath, the epineurium, was not removed, anesthetics were found to be more effective in alkaline solutions (32, 43, 61, 75, 78, 103). As described above, this finding can be readily explained in terms of either the cation or the uncharged molecule being the active form. On the other hand, in most studies where the nerve sheath was removed, anesthetics were more effective in neutral than in alkaline solution (29, 72, 74, 75). This finding can be readily explained on the theory that the cation is the active form, but not feasibly on the basis of the uncharged molecule being active. The difference between sheathed and desheathed nerves can readily be seen in the same preparation (75). The situation that prevails in a sheathed nerve is illustrated diagrammatically in Figure 2. The anesthetic crosses the sheath in the uncharged form. Once inside the sheath, the uncharged molecule enters a relatively neutral environment, regardless of the external pH, because of the action of tissue buffers. A large proportion of the uncharged molecule is then converted back to the cationic form, which in turn reacts with the receptor to block conduction. In this way, both the concentration of anesthetic at the receptor, and the rate of increase in this concentration, will be determined by the concentration of uncharged molecule in the external medium which, with a given concentration of local anesthetic, will be greater when the solution is alkaline.

It is interesting that if the pH in the periaxonal space (i.e., inside

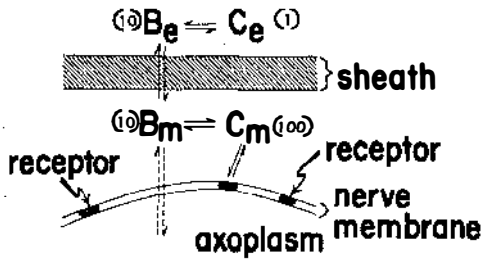


FIG. 2. The distribution of the basic (B) and cationic (C) forms of a local anesthetic in a sheathed nerve. The subscript $_e$ denotes the concentration in the external bathing fluid and the subscript $_m$ that in the immediate vicinity of the receptors in the membrane. The numbers within the parentheses represent the concentrations of the individual constituents, B_e, C_e, B_m, and C_m, under the following conditions. The total external concentration of anesthetic is 11 units; the external pH is 9.7; the pH in the immediate vicinity of the receptors is 7.7; the pK_a of the local anesthetic is 8.7. Penetration of the nerve sheath by the uncharged molecule, interaction of the cation with the receptor, and penetration of the axoplasm by the uncharged molecule are also illustrated.

the epineurium) is less alkaline than the pH in the bulk of the external solution, the system actually concentrates the local anesthetic in the region of the receptors. This increase is entirely in the form of the cation and is determined by the relation $\log C_m/C_e = pH_e - pH_m$, the subscripts $_e$ and $_m$ referring to the external solution and the solution next to the membrane, respectively.

A critical assumption in the foregoing discussion is that the main external nerve sheath, the epineurium, presents a substantial barrier to diffusion of the local anesthetic in its cationic form. This has recently been questioned by Honrubia & Lorente de Nó (53) who reported that at pH 7.3, 3 mM lidocaine applied to a frog single myelinated fiber, which is, of course, desheathed, produced the same rate of "block" as 7 mM lidocaine applied to a sheathed nerve, which would seem to support the idea of the sheath being a diffusion barrier. However, upon removal to anesthetic-free Ringer's solution, the intact nerve was reported to "recover" more rapidly than the single fiber. From this and other results they concluded that the sheath does not present the sort of diffusion barrier to local anesthetics postulated above. However, it is doubtful whether any valid conclusion about the relative sensitivity of sheathed and desheathed fibers can be drawn from these results because disparate methods of electrical recording were used in the two situations. The method used for the intact nerve is not stated, but presumably the authors recorded the size of the conducted action potential after it had propagated for some distance from the stimulating electrodes, using a method similar to that described by Lorente de Nó (62). On the other hand,

the experiments on the desheathed nerve were made using the bridge insulator technique, in which electrical records are obtained from the same node that is being electrically stimulated. The "action potential" recorded under these latter conditions need not, and often does not (53), propagate to the next node; nor, as Honrubia & Lorente de Nó stress, does such an "action potential" obey the all-or-none law. Furthermore, Honrubia & Lorente de Nó state that an "action potential" could always be obtained by them in sheathless nerves even in the presence of concentrations of anesthetic sufficiently large "to cause irreversible deterioration of the nerve fibers." This sharply contrasts with the universally accepted view that local anesthetics do, in fact, block conduction of the propagated action potential. It can be misleading, therefore, to compare the results of experiments on sheathed nerves in which the index of activity must have been a propagated all-or-none spike, with experiments on single nerve fibers in which the "action potential" is usually not propagated, is not all-or-none, and is relatively insensitive to local anesthetics.

The influence of the perineurium and endoneurium.—The sheath depicted in Figure 2 need not be the epineurium. In the case of myelinated nerve fibers, the perineurium and endoneurium appear to play a similar role. Thus, Skou (86), who worked with frog sciatic nerves from which the epineurium had been removed, found that local anesthetics were more effective in alkaline solutions. From an analysis of Skou's data, Ariëns & Simonis (7, 8) concluded that the pH in the immediate vicinity of the receptors did not change to the same extent as the pH in the bulk of the solution because of the presence of a tissue barrier. Assuming that a constant amount of cation in the immediate vicinity of the receptor is necessary to produce anesthesia, they calculated from Skou's data on procaine that the pH in this region changed from 6.92 to 7.64 when the pH in the external medium changed from 6.0 to 8.0. Using this calculated relation between the external pH and that in the region of the receptors, they then calculated, from the data on the minimum effective concentration of the four other anesthetics used by Skou (cocaine, tropacocaine, tetracaine, and dibucaine), the concentration of cation and of uncharged molecule near the receptors at the different values of pH. The results clearly indicated that for each anesthetic the concentration of ionized drug in the biophase remained reasonably constant whereas that of the uncharged molecule did not. The consistency of their calculations thus lends strong support both for the assumption of a permeability barrier to hydrogen and other cations around the nodal membrane, and for the conclusion that the cation is the active form of local anesthetics.

The presence of such a barrier is independently suggested by the absence of any action of acetylcholine on desheathed preparations of myelinated fibers (96), although acetylcholine seems to have a well defined action on other axons, such as mammalian nonmyelinated fibers (9, 10, 70,

71), the somatic nerves of the lobster (28), and squid giant axons after their permeability barriers have been weakened by snake venoms (76, 77).

Relative roles played by uncharged molecule and cationic form in local anesthetic action.—In summary, the theory that the uncharged molecule is the active form cannot account for the results of the experiments with desheathed nonmyelinated fibers; nor can it account satisfactorily for the results of experiments on intact preparations because the observed slope of the line relating minimum effective concentration to pH is not the same as the theoretical curve. On the other hand, the theory that the cation is the active form of the anesthetic is consistent with all the known effects of local anesthetics on nerve. These are: the effect of pH on the minimal effective concentration in equilibrium conditions [(43, 78, 86, 103); see analysis of Ariëns & Simonis in (7, 8)]; the effect of pH on desheathed nerves during the period of recovery from local anesthesia (72, 74); the effect of pH on the action of local anesthetics on frog single nerve fibers (29); the effect of pH on the penetration of nerves by local anesthetics (74); the effect of pH on the rate of block in desheathed nerves (74); and, finally, a comparative study of the effect of pH on the action of local anesthetics in sheathed and desheathed preparations (75). The findings in these six different types of experiments are all readily explicable on the assumptions that the base is the important form for penetration and that the cation is the active form at the receptor in the nerve membrane.

The idea that local anesthetics penetrate tissues as uncharged molecules and act at the membrane as cations provides an explanation for the fact that nearly all of the most effective local anesthetics are amines with pK_a values lying within a fairly narrow range. The pK_a values of these common local anesthetics lie in a range such that appreciable quantities of both the uncharged and cationic forms exist at physiological pH values. A quaternary ammonium compound, or indeed any basic compound with too high a pK_a, would be expected not to be an effective local anesthetic because it should not be able to reach the conducting system in the nerve membrane in sufficient quantities at the physiological pH. On the other hand, any base with too low a pK_a, although it might penetrate the nerve fibers readily, should also be ineffective because of the relatively small amount that would exist in the cationic form at the site of action in the nerve fibers.

An important consequence of the demonstration that local anesthetics act in their cationic form is that the general and the nitrogenous local anesthetics must block the conduction of the action potential by somewhat different mechanisms. For, although central nervous depressants such as barbiturates, chloroform, and alcohols can block impulse conduction in nerve and muscle apparently, as do the nitrogenous local anesthetics, by reducing the transient increase in sodium conductance that generates the action potential (79, 100), these agents cannot exist as cations. That local and general anesthetics have a different mechanism of action is further sug-

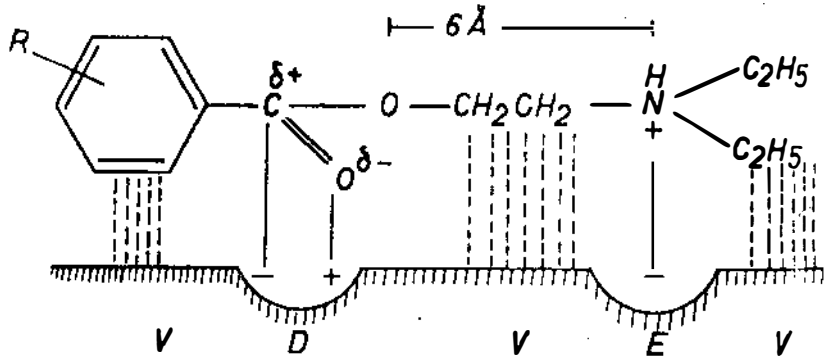
gested by the fact that, unlike the local anesthetics, chloroform and many of the alcohols depolarize the nerve membrane in the concentrations required to produce nerve block (69).

Relations Between Structure and Activity

The relations between molecular structure and pharmacological activity have probably been studied more extensively for local anesthetics than for any other group of pharmacologically active compounds. The reasons for this are presumably to be found in the relatively simple structures involved and in the ease and quantitative nature of the tests for local anesthetic action. A large number of such tests have been performed, and it is important to distinguish between those measurements of local anesthetic potency useful in evaluating the potential clinical effectiveness of an agent, and those which measure its intrinsic potency, i.e., its true potency when in direct contact with the nerve membrane. It is difficult, and often impossible, to determine the relation of structure to true potency from the results of tests on complex preparations, such as the rabbit's cornea, which are affected by many other factors such as diffusion, tissue binding, and metabolism. For example, Büchi & Perlia (24) concluded, from an investigation of the ability of dibucaine and its homologues to anesthetize the rabbit's cornea, that the relative pharmacological activity of the members of the series was largely determined by their ability to reach the nerve membrane, which in turn depended on physicochemical factors such as lipid and water solubilities. Despite the many factors that influence most tests for local anesthetic potency, it has been possible through the study of the relation of chemical structure and biological activity to obtain some understanding of the structural requirements both for clinical usefulness and for high intrinsic potency.

Löfgren (61) concluded that, with few exceptions, the useful local anesthetics are composed of three constituents: (*a*) a carbocyclic or heterocyclic ring of aromatic type (lipophilic portion), (*b*) an intermediate chain, and (*c*) an amino group (hydrophilic portion). An enormous variety of structural modifications within this general pattern is effective in producing conduction block in nerve fibers. An exhaustive review of structure-activity relations for local anesthetics would thus require many hundreds of pages. However, in the present review, the discussion of structure-activity relations will be restricted to certain of the aspects that shed light on the possible mode of action of local anesthetic molecules.

From a study of the relation between chemical structure and local anesthetic activity, Büchi & Perlia (23) formulated a hypothesis concerning the nature of the binding of local anesthetic molecules to receptors in the nerve membrane. They suggested that the drug is bound to the receptor by three types of forces, namely, van der Waals forces, dipole-dipole interactions, and electrostatic binding (Fig. 3). A theoretical electron analysis



. FIG. 3. Model of binding of local anesthetic molecule to the receptor by: V, van der Waals forces; D, dipole-dipole interaction; E, electrostatic forces. Taken from Büchi & Perlia (23).

by Löfgren (61) had indicated that all effective local anesthetic compounds in the carboxylic acid ester and amide series are characterized by a highly reactive carbonyl group, in which the electron cloud at the oxygen atom is sufficiently dense to act as an electron donor capable of forming hydrogen bonds. Büchi & Perlia have provided support for, and have extended, this hypothesis, finding that the more potent local anesthetics have a greater negativity associated with the oxygen atom of the carbonyl group. For example, substitution of $-NH_2$, $-NHR$, $-OH$, or $-OR$ groups in the para position of diethylaminoethyl benzoate results in a partial shift of electrons along the conjugated carbon series, concentrating electrons at the carbonyl oxygen (Fig. 4), and increasing the local anesthetic potency.

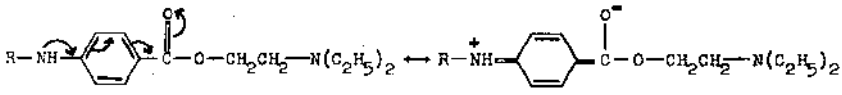


FIG. 4. Resonance forms of a local anesthetic molecule. The arrows indicate the electron shifts.

The theory readily accounts, for instance, for the local anesthetic activity of procaine (p-NH_2 group), tetracaine (p-C_4H_9-NH group), and pramoxine (p-C_4H_9O group) where the introduction of an electron-donating substituent results in a marked intensification of the weak effects of the parent compounds. On the other hand, electron-withdrawing substituents, such as the $-NO_2$ group, result in the opposite effect, with regard both to electron distribution and to local anesthetic action, as illustrated by diethylaminoethyl paranitrobenzoate, which has negligible local anesthetic action. In general, therefore, compounds of the type illustrated in Figure 4, in which resonance effects result in an active anionic carbonyl oxygen, and particularly those in which the negativity of the carbonyl oxygen is partially

intensified by electron-donating substituents, are potent local anesthetics. On the other hand, compounds without such resonance effects, and compounds with resonance effects but where the negativity of the carbonyl group has been diminished by adding electron-withdrawing substituents to the benzene ring, are poor local anesthetics.

The effects of modifying the intermediate chain of the local anesthetic molecule on its potency can also be explained in terms of effects on the electron distribution around the carbonyl oxygen. For example, addition of either one or two methyl groups to the α-carbon of the intermediate chain produces a shift of electrons from the α-carbon towards the carbonyl group, makes the electron cloud of the carbonyl oxygen more dense, and results in an intensified local anesthetic activity.

Similar considerations to those just described apply equally well when the -O- of the ester series is replaced by the -NH- of the amide series. In this latter series, intensified local anesthetic activity is again associated with an increased electronegativity of the carbonyl oxygen. From these and other considerations, Büchi & Perlia (23) concluded that the aromatic moiety of a potent local anesthetic molecule subserves several functions: it provides adequate lipid solubility of the local anesthetic; it allows sufficient capacity for receptor binding by van der Waals forces; and, finally, it participates in the establishment of a strong anionic reaction site in the carbonyl group of the intermediate chain. Analysis of other types of structures used as local anesthetics (23, 61) indicates that practically all of them similarly contain a polarizable dipole approximately two or three carbon atoms distant from the ionizable amino group.

Further evidence that the electronic structure of the ester group of a local anesthetic molecule is of paramount importance in determining its potency has been provided by Galinsky et al. (42), who used the infrared carbonyl absorption frequency as a measure of the reactivity of the carbonyl group in a series of diethylaminoethyl para-substituted benzoates, cinnamates, and β-phenyl propionates. In general, a good correlation existed between the infrared spectra and the local anesthetic potency.

The importance of the intramolecular charge distribution of the molcule in determining the potency of local anesthetics is clear from the experiments discussed in this section. However, it should be emphasized that other structural aspects of the local anesthetic molecule can be extremely important both for their intrinsic potency and for their clinical efficacy. For example, the excellent anesthetic properties of lidocaine are largely caused by the fact that the methyl groups in the ortho positions of the benzene ring prevent, by steric hindrance, the rapid hydrolysis of the amide group (61). However, the present discussion has been deliberately slanted to make one important point, namely, that the studies on the relations between chemical structure and biological activity are beginning to suggest a definite molecular arrangement not only for the local anesthetic molecule, but also for the receptor in the nerve membrane with which it reacts. Such

knowledge, which could not easily come from purely electrophysiological or electropharmacological studies, is clearly essential to a final understanding of the mode of action of local anesthetics.

Model Systems

It has become increasingly clear in recent years that living cells exploit the spontaneous property of surface-active lipids, particularly phospholipids, of orienting themselves in the form of bimolecular sheets or membranes. With this recognition, the question has arisen whether the semipermeability and electrical characteristics of conducting cells can be explained to any extent in terms of the physical chemistry of lipid films. A secondary question, more germane to the present review, has also arisen, namely, whether drugs that affect conduction, such as local anesthetics, act by a simple disturbance of some physicochemical property of the lipid membrane surrounding the cell. For this reason, many studies have been made of the effects of local anesthetics on isolated lipid model systems. Some striking correlations have been found between the ability of local anesthetics to block conduction of the nerve impulse and their effects on these model systems, particularly on lipid monolayers at air/water interfaces and on lipid membranes separating two aqueous phases. These will now be discussed.

Lipid monolayers at an air/water interface.—Skou examined the ability of five local anesthetics and of butanol to penetrate monomolecular layers of stearic acid (89) and of an extract of nerve tissue lipids (90). The extent of penetration of the monolayers was determined from measurements of the increase in surface pressure. He found that the relative blocking potency of each of the six compounds studied was approximately equal to its relative ability to penetrate monomolecular layers of nerve tissue lipids. This result is particularly impressive in view of the fact that the blocking potencies of the compounds tested varied by a factor of more than ten thousand. Moreover, the ability to penetrate a monolayer of nerve tissue lipids depended on pH in a manner similar to the ability to block nerve conduction (90). These results suggested to Skou that the site of blocking action of local anesthetics is at a lipid-containing interface, and that the blocking effect may be caused by physical changes produced by the penetration. Later, Skou (93) demonstrated that the close parallelism between blocking potency and ability to penetrate monomolecular layers of nerve lipids extends to thymol, β-naphthol, menthol, and a series of aliphatic alcohols.

Subsequently, Shanes & Gershfeld (84) found a number of effects of procaine and veratrum alkaloids on monomolecular films of stearic acid that correlated with their effects on nerve fiber membranes. They confirmed Skou's findings (89) that local anesthetics cause an increase in surface pressure of the stearic acid monolayer. However, they made several additional important observations. They investigated four veratrum alkaloids, veratridine, cevadine, veracevine, and veratramine. Veratridine and cevadine were shown to cause a gradual decrease in surface pressure, ap-

parently owing to a withdrawal of stearic acid from the monolayer. This action of the veratrum alkaloids, opposite to that of the local anesthetics, thus correlates well with the effect of the alkaloids on nerve membrane in being opposite to that of the local anesthetics; local anesthetics decrease permeability to sodium and potassium ions whereas the veratrum alkaloids increase permeability to these cations. Moreover, the concentrations of veratridine and cevadine that were active on the monolayers were found to be the same as those active on the nerve membrane. Veracevine, which is inactive compared to veratridine and cevadine on nerve membranes, was also ineffective on the monolayers. Veratramine, which has a twofold action on the nerve membrane, decreasing permeability at low concentrations and increasing it at high concentrations, also had a twofold action on the stearic acid monolayers, causing an increase in surface pressure at low concentrations and a decrease in surface pressure at high concentrations. Finally, and perhaps most convincing of all, the effects of veratridine and cevadine on the stearic acid monomolecular layers could be antagonized by three different procedures that also antagonize the action of these alkaloids on the nerve membrane, namely, the addition of 10^{-3} *M* calcium, low pH, and treatment with procaine.

Gershfeld (44) has found a certain parallelism between the pharmacological effects of veratrine and procaine and the effects of these drugs on the desorption kinetics of a monolayer of mono-octadecyl phosphate. Thus, veratrine, which increases ion permeability of the nerve membrane, was found to disrupt the alkyl phosphate monolayers, whereas procaine, which decreases ion permeability of the nerve membrane, was found to counteract film instability of the monolayer.

On the basis of the studies of monolayers (84, 89, 90, 93) Shanes has proposed (81, 83) that local anesthetics block impulse conduction by virtue of increasing the lateral pressure of the lipid layer that constitutes the nerve membrane, with a resultant occlusion of the pores through which sodium and potassium ions move. This explanation for the mode of action of local anesthetics, which is essentially a mechanical one, has been criticized by Bangham (11). One of Bangham's arguments is that the lipid molecules in the membrane are not restrained by lateral barriers, and therefore adsorption of further molecules is likely to be isopiestic. Bangham has suggested that compounds that stabilize the nerve membrane are more likely to act by modifying the compositional mosaic of lipids, thereby enhancing their bimolecular structure.

Lipid membranes separating two aqueous phases.—Recently, model systems have been used that involve lipid membranes separating two aqueous phases. Such systems would seem to be better models of the nerve membrane, which is a bimolecular membrane separating two aqueous phases, than are the monomolecular layers described in the previous section, which separate a single aqueous phase from air.

Feinstein (38) studied the effect of local anesthetics on lipid-impregnat-

ed Millipore filters of the type described by Tobias, Agin & Pawlowski (102). In contrast to the monolayer model systems just described, these lipid membranes permit the measurement of certain of the parameters measured in nerve, such as electrical conductance. As described earlier, local anesthetics decrease the electrical conductance of nerve, the sodium conductance being more affected than the potassium conductance. It is interesting, therefore, that Feinstein found that 3 m*M* tetracaine decreased the conductance of the Millipore filter when potassium, and even more so when sodium, was the cation in the solution on both sides of the artificial membrane. The local anesthetics possibly achieve these effects on conductance in the model membrane and in the nerve membrane by forming a complex with a phospholipid that ordinarily serves as a carrier for transporting potassium and sodium ions. Feinstein found a variety of other types of evidence to support the suggestion of complex formation between local anesthetics and phospholipids. Thus, local anesthetics inhibit the phospholipid facilitated transport of calcium into chloroform, they coagulate aqueous dispersions of phospholipid stoichiometrically (one mole of local anesthetic forming a complex with two moles of cephalin), and they displace hydrogen ions from cephalin sols. As in nerve (see above), the effects of local anesthetics in the various phospholipid systems could be mimicked by calcium ions, which decrease the conductance of lipid-impregnated Millipore filters, coagulate phospholipids, and displace hydrogen ions from aqueous dispersions of cephalin. Feinstein's data indicated the cation, rather than the uncharged molecule, to be the form in which local anesthetics inhibit calcium-binding by phospholipids. He proposed (38) that complex formation between the local anesthetics tested and the phospholipid involves the formation both of an ion-ion bond between the positively charged tertiary alkyl nitrogen and the phosphate moiety of one phospholipid molecule, and of an ion-induced dipole bond between the nitrogen in the para position of the benzene ring and the phosphate moiety of a second phospholipid molecule.

Another extremely promising model for the nerve membrane is the system of phospholipid lamellae developed by Bangham (11). These lamellae are liquid crystals of lecithin that form spontaneously when the lipid is placed in a salt solution. They consist of a concentric series of roughly spherical, bimolecular sheets of lipid, each separated by an aqueous phase. The fluxes of ions in and out of this system can be readily measured (13). Preliminary experiments (12) indicate that local anesthetics slow the loss of cation from this system, reminiscent of the effect of local anesthetics in nerve. The charged form of the local anesthetic molecule seems to be the active one. Further experimentation with this interesting model should prove rewarding.

Conclusion

It is, of course, extremely difficult to obtain unequivocal evidence in support of one or another theory for the mechanism of local anesthesia.

The known effects of local anesthetics on the nerve membrane can be explained equally well on the basis either of a pore theory or of a carrier theory for ion movements. Nevertheless, it does seem possible to draw some fairly definite conclusions concerning the form in which, and the site at which, local anesthetics act. Thus, a variety of evidence, reviewed in the present paper, strongly suggests that local anesthetics act in their cationic, rather than their uncharged, form at or in the nerve membrane, through modifying the physicochemical state of its lipid constituents. The result is an alteration of ion permeability followed by conduction block. The precise alteration in lipid structures that brings about these changes remains unknown. However, promising model systems that might help to elucidate the structural changes are now available (13, 102). These new models, involving two aqueous phases separated by a lipid membrane, would seem to provide a better model of the nerve membrane than do the monomolecular layers previously studied. In these new systems the electrical conductance, which could not be measured in the monolayer model, is decreased by local anesthetics (38), as in the living nerve. Furthermore, in contrast to the monolayer model (81, 89, 90), and in agreement with recent electrophysiological studies on the living membrane (7, 8, 29, 72, 74, 75), the cation rather than the uncharged molecule is the active form of the local anesthetic in both of the new lipid membrane models (12, 38).

Clearly, the major limitation in our understanding of the mode of action of local anesthetics in blocking impulse conduction is our lack of knowledge of the excitable process itself, and of the mechanism by which the transient depolarization of the membrane is propagated along the nerve fiber. Electrophysiological work in recent years has provided us with a thorough understanding of the ionic basis of impulse conduction. However, the underlying mechanism by which the changes in ion permeability are brought about is completely unknown, and it is manifestly impossible to pinpoint the mode of action of local anesthetics until we have attained a better understanding of the factors involved in the permeability changes. Critical studies of the action of local anesthetics on nerve may well be one of the methods whereby this understanding of the underlying physiological processes is achieved.

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