2

Pharmacodynamic Aspects of Local Anesthetic Agents

ANATOMY OF PERIPHERAL NERVE

Peripheral nerves are mixed nerves containing both sensory afferent fibers and motor efferent fibers. Figure 2-1 presents a cross-sectional view of a typical peripheral nerve and illustrates some of the anatomical factors that influence the pharmacological properties of local anesthetic agents. Peripheral nerves possess a distinctive organizational pattern. Each individual axon is surrounded by a connective tissue sheath known as the endoneurium. Groups of axons are enclosed in an additional connective tissue sheath called the perineurium. Finally, a number of axonal groups are encased in an external connective tissue sheath, the epineurium. Interference with the conduction process by pharmacological means requires diffusion of chemical compounds through these connective tissue layers to the individual axonal fiber. Thus, the epineurium, perineurium, and endoneurium are considered anatomical barriers to the diffusion of local anesthetic substances. Differences exist with regard to the rate at which various local anesthetic agents diffuse through these connective tissue sheaths. The physicochemical properties of individual compounds themselves and the physiological state of the local milieu surrounding the nerve fibers will determine the rate of diffusion and ultimately the onset of analgesia.

A consideration of the morphological features of the axon itself also reveals certain barriers that may influence the movement of local anesthetic agents. Figure 2-2 presents a diagrammatical cross-sectional sketch of a myelinated and unmyelinated nerve. An un-

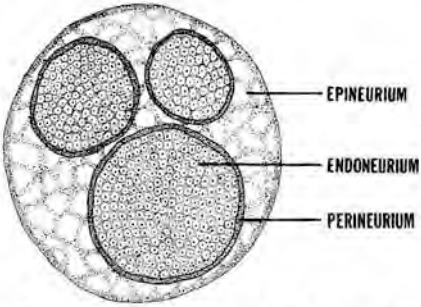


Fig. 2-1. Cross-sectional diagram of a peripheral nerve.

myelinated nerve fiber is surrounded by a single wrapping, the Schwann cell sheath. Groups of unmyelinated fibers share the same Schwann cell. All postganglionic fibers of the autonomic nervous system and fibers less than 1 micron in diameter (C fibers) in the somatic nervous system are unmyelinated, e.g., pain fibers and motor fibers to involuntary muscles.

Myelinated fibers are enclosed in spirally wrapped layers of lipoprotein myelin sheaths which are actually a specialized form of a Schwann cell. Each myelinated fiber is enclosed in its own myelin sheath. The outermost layer of myelin essentially consists of the Schwann cell cytoplasm and its nucleus. The myelin sheath surround-

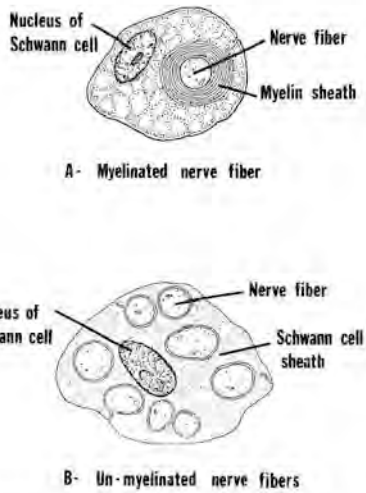


Fig. 2-2. Comparative morphology of myelinated and unmyelinated nerves.

ing a myelinated nerve fiber (A & B fibers) is interrupted at intervals by constrictions, i.e., the nodes of Ranvier. The size of the individual Schwann cell determines the distance between adjacent nodes. These myelin sheaths are essentially lipid in nature (75%), but also contain some protein (20%) and carbohydrate (5%) material.[30] The molecular structure of these myelin sheaths has been described by various investigators as consisting of two bimolecular leaflets of lipid with polar heads pointing outward and each polar surface covered by a monolayer of protein.[31–33] However, the precise molecular configuration of this lipoprotein membrane is still a matter of some debate.

In addition to the connective tissue and myelin, or Schwann cell sheath, enveloping nerve fibers, the neuronal axon possesses its own cell membrane, the axolemma, which surrounds the axoplasm. Various molecular structures have been proposed for biological membranes including the axonal membrane. Danielli and Davson originally suggested that the cell membrane consisted of a lipid core arranged in such a fashion that the polar lipid heads were oriented in an outward direction and were covered by a nonlipid monolayer which was probably protein in nature.[34] Several modifications of this basic model have been proposed.[35–37] In 1959, Robertson advocated the term "the unit membrane" as the fundamental unit of all biological membranes.[33, 36] The unit membrane was visualized as consisting of a bimolecular leaflet of unspecified lipids at the center with the polar lipid heads pointed outward and covered on both exposed surfaces by a monolayer of nonlipids. This model differed from that of Danielli and Davson in that the nonlipid layers covering the internal and external surface of the polar lipid heads were believed to differ in chemical composition. Although the exact structure and chemical composition of the unit membrane may not be accepted universally, it is generally believed that biological membranes do consist essentially of a lipoprotein matrix arranged in a fashion somewhat similar to that shown in Figure 2-3.

More recent membrane models visualize proteins rather than lipids as the basic organizational elements of membranes.[38–40] Although the importance of lipids as an essential part of the membrane is still appreciated, current models describe the membrane as being heterogenous in nature with a greater interaction between protein and lipid molecules such as shown in Figure 2-4. A comparison of various models depicting the molecular structure of the cell membrane is presented in Figure 2-5.[41]

Although the axolemma was known to consist mainly of lipids and to a lesser degree protein and carbohydrates, the exact composi-

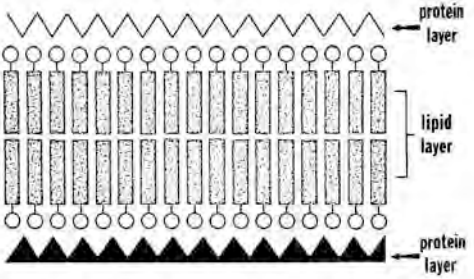


Fig. 2-3. Unit membrane as suggested by Robertson.[36]

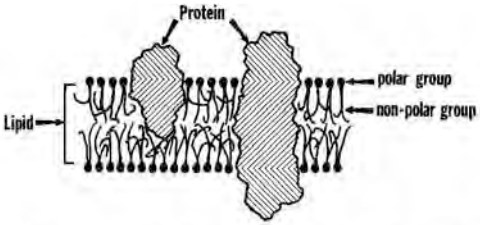


Fig. 2-4. Heterogeneous lipoprotein membrane as suggested by Singer.[40]

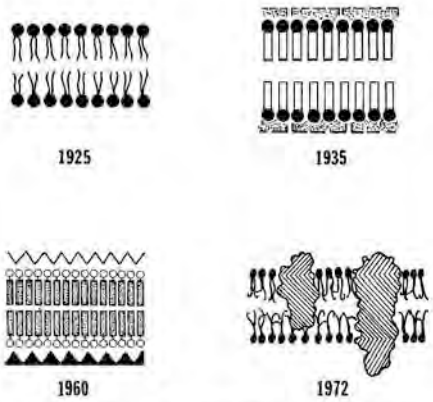


Fig. 2-5. Comparison of various cell membrane models suggested between 1925 and 1972.

tion was not well known. The most complete chemical analysis of the axolemma was accomplished by Camejo and co-workers in 1969.[42] Utilizing the first stellar nerve of the giant squid, these authors extruded the axoplasm and subjected the remaining material to centrifugation. Two fractions were obtained in this manner. That fraction possessing the greater enzymatic activity was assumed to be the axolemma and contained both proteins and lipids. The protein to lipid ratio for the squid axolemma was 0.13, indicating that the greatest portion (almost 90%) of the axolemma consisted of lipids, mainly, phospholipids such as phosphatidyl choline, phosphatidyl ethanol amine, phosphatidyl serine, and sphingomyelin (Table 4).

Irrespective of the precise structure and composition of the nerve membrane, it is apparent that lipids and proteins play an essential role in the molecular organization of these membranes. This suggests that the physicochemical properties of local anesthetic agents, namely lipid solubility and protein-binding, are important in terms of the ultimate interaction between specific local anesthetic drugs and the nerve membrane. This interrelationship between the physicochemical properties of specific agents, the structure of the nerve membrane, and the resultant biological action, can be demonstrated if one compares certain homologous local anesthetic compounds. For example, lidocaine and etidocaine are related amide-type local anesthetic agents that possess similar pK_a values, but differ markedly in their lipid solubility and protein-binding characteristics. A comparison of the biological effect of these two agents on the isolated frog sciatic nerve reveals that etidocaine has a more rapid onset of conduction

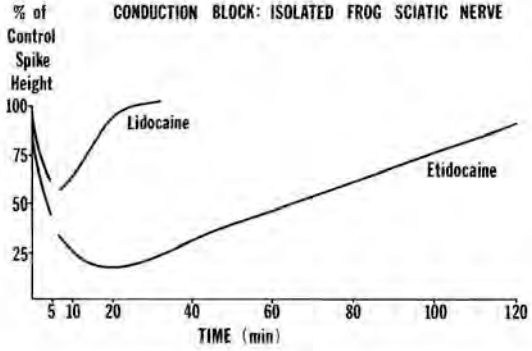
Table 4

PERCENTAGE WEIGHT COMPOSITION OF MEMBRANE FRACTIONS ISOLATED FROM GIANT SQUID NERVE

PROTEIN	29.5 ± 1.4
TOTAL LIPIDS	70.5 ± 1.5
CHOLESTEROL	28.1 ± 2.3
FATTY ACIDS	6.2 ± 0.9
POLAR LIPIDS	58.5 ± 3.5
PHOSPHATIDYL CHOLINE	45.9 ± 2.9
SPHINGOMYELIN	10.0 ± 1.6
PHOSPHATIDYL ETHANOLAMINE	34.4 ± 1.7
PHOSPHATIDYL SERINE	10.4 ± 2.3

Data derived from Camejo et al (1969)

blockade, a greater potency, and a longer duration of action.[43] These biological effects are consistent with the greater lipid solubility of etidocaine, which suggests that it should diffuse through the lipid myelin sheath and axonal membrane more easily and so have a shorter latency. The greater protein-binding capacity implies that it should bind to a greater degree with the protein component of the membrane and, thus, be an agent of greater intrinsic anesthetic potency and longer duration of action (Fig. 2-6). The basic relationship



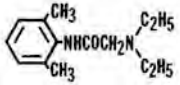
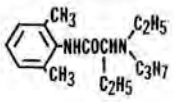
	LIDOCAINE	ETIDOCAINE
Chemical Structure		
pK_a	7.72	7.74
Partition Coefficient	3.6	142
Protein binding	55%	94%
Relative anesthetic potency	1	4

Fig. 2-6. Relative physicochemical and conduction-blocking properties of lidocaine and etidocaine.

between nerve morphology, chemical structure, and physicochemical properties of local anesthetic compounds may be obscured under clinical conditions due to other biological factors. However, ulnar nerve block studies in man, comparing lidocaine and etidocaine, reveal results similar to those obtained on the isolated frog sciatic nerve, which suggest that these fundamental anatomical-chemical-biological interrelations can be demonstrated clinically (Fig. 2-7). [44]

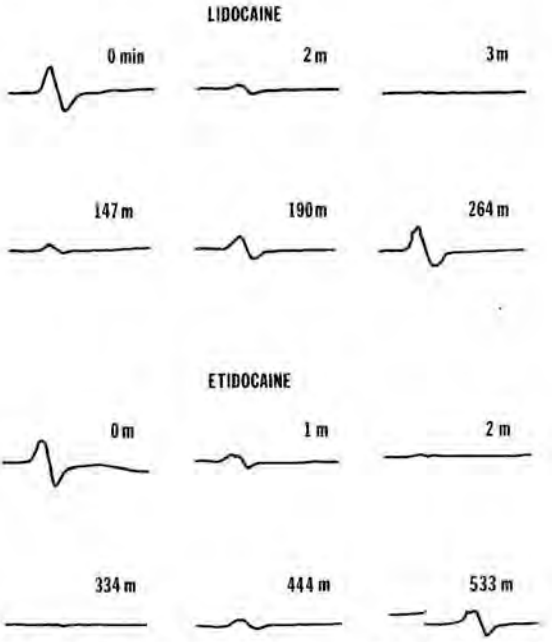


Fig. 2-7. Hypothenar electromyographs prior to and following ulnar nerve block with 1% lidocaine and 0.5% etidocaine.

PHYSIOLOGY OF PERIPHERAL NERVE

Membrane Electrophysiology

Local anesthetic agents exert their primary pharmacological action by interfering with the excitation-conduction process of peripheral nerve fibers and nerve endings. The development of microelectrodes that could be inserted intracellularly has made possible biophysical studies that have provided information concerning the basic electrophysiological properties of nerve tissue.[45] Figure 2-8 graphically depicts the transmembrane action potential of the peripheral nerve as recorded by such an intracellular electrode.[44]

During the period of nerve inactivity, a negative electrical potential (resting potential) of approximately −60 to −90 mv exists across the cell membrane. When excitation occurs, a consistent sequence of events takes place. An initial phase of depolarization is observed during which the electrical potential within the nerve cell becomes

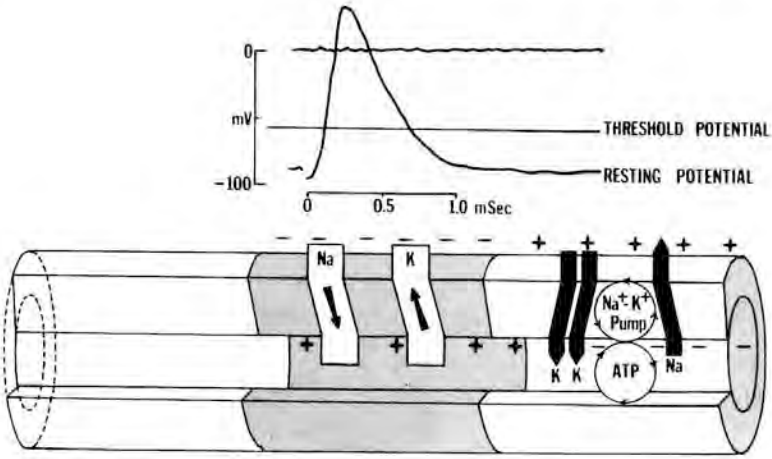


Fig. 2-8. Relationship between membrane action potential and ionic flux across the nerve membrane.

progressively less negative. When the potential difference between the interior and exterior surface of the cell membrane reaches a critical level, the threshold potential, or firing level, an extremely rapid phase of depolarization commences which results in a reversal of the electrical potential across the cell membrane, such that the inside of the membrane becomes positively charged with respect to the outside of the cell membrane. At the peak of the action potential, the interior of the cell has a positive electrical potential of approximately +40 mv as compared to the exterior of the cell. After completion of the depolarization phase, repolarization begins, and during this time, the electrical potential within the cell again becomes progressively more negative with respect to the exterior of the cell until such time as the resting potential of −60 to −90 mv is reestablished. Under normal conditions, this entire process of depolarization and repolarization occurs within 1 msec. The depolarization phase occupies approximately 30% of the entire action potential whereas repolarization accounts for the remaining 70%.

Membrane Electrochemistry

The electrophysiological properties of the nerve membrane are dependent on (a) the concentration of electrolytes in nerve cytoplasm and extracellular fluid and (b) the permeability of the cell membrane to various ions—particularly, sodium and potassium. The ionic composition of the cytoplasm and the extracellular fluid differ markedly.

The intracellular concentration of potassium is approximately 110 to 170 meq/l, whereas the intracellular concentration of sodium and chloride ions is approximately 5–10 meq/l. In extracellular fluid, the situation is reversed. The concentration of sodium is approximately 140 meq/l and the concentration of chloride is 110 meq/l, whereas the extracellular concentration of potassium is 3–5 meq/l.

This ionic asymmetry on either side of the cell membrane is due in part to the selective permeability characteristics of the membrane. The resting membrane is fully permeable to potassium ions, but only slightly permeable to sodium ions, which accounts for the low intracellular concentration of sodium. The high intracellular concentration of potassium is maintained by the attractive forces of the negative charges, mainly on proteins, within the cell which counterbalances the tendency of potassium ions to diffuse out of the cell by passive movement along a concentration gradient and across a freely permeable membrane. Nernst derived an equation to predict the electrical potential across a membrane separating two concentrations of the same ion:

$$E = -\frac{RT}{nF} \ln \frac{[A]_i}{[A]_o}$$

E = membrane potential between inside and outside of the cell
R = gas constant in joules (8.315)
T = absolute temperature
n = valence of the ion
F = Faraday's constant (96,500 coulombs)
ln = natural logarithm

At room temperature (18°C) and assuming a K_i/K_o ratio across the nerve membrane of 30, i.e., $[150 \text{ meq/l}]_i/[5 \text{ meq/l}]_o$, the Nernst equation would predict the following:

$$E = -58 \log \frac{[30K]_i}{[K]_o}$$

$$E = -85.7 \text{ mv}$$

This predicted resting membrane potential of −85.7 mv agrees closely with the commonly reported values of −60 to −90 mv measured directly in nerve preparations with intracellular electrodes. Thus, at rest, the nerve cell behaves as a potassium electrode that would react to intra- or extracellular changes in potassium concentrations, but not sodium concentrations. Indeed, the resting membrane

potential of nerves can be altered by changes in the potassium content of extracellular fluid, but is unaffected by variations in the sodium concentration.[46]

Attempts have been made to utilize clinically the relationship between the resting potential and the K_i/K_o ratio. Thus, potassium chloride has been added to solutions of local anesthetic agents in an attempt to combine the conduction blocking effects of a reduced resting potential and an anesthetic drug. The duration of action and quality of anesthesia produced by procaine can be increased by the concomitant use of KCl in a concentration of 135–150 m*M*.[47] The addition of KCl to solutions of lidocaine has been reported to decrease the onset time and improve the quality of epidural anesthesia[48] and to prolong the duration of digital and ulnar nerve blocks in man.[49]

Membrane Activation

Excitation of a nerve results in an increase in the permeability of the cell membrane to sodium ions. The initial flux of sodium ions from the exterior of the cell membrane to the interior of the nerve cell results in a depolarization of the cell membrane from the resting potential level to the threshold or firing level of approximately −50 to −60 mv. At this point, a maximum increase in the permeability of the cell membrane to sodium ions occurs and an explosively rapid influx of sodium ions into the axoplasm follows. At the end of depolarization or at the peak of the action potential, the nerve membrane is essentially transformed from a potassium electrode to a sodium electrode and the positive membrane potential of +40 mv can be calculated again from the Nernst equation by substituting the ratio of sodium ions between the inside and outside of the nerve membrane (Na_i/Na_o) for the potassium ion ratio (K_i/K_o).

At the conclusion of the depolarization phase, the permeability of the cell membrane to sodium ions again decreases and high K^+ permeability is restored. Potassium moves out of the cell, resulting in repolarization of the membrane until such time as the original electrochemical equilibrium and resting potential is reachieved. The flux of sodium ions into the cell during depolarization and potassium ions out of the cell during repolarization is a passive phenomenon, since each ion is moving down its concentration gradient.

The relationship of the membrane potential to the potassium and sodium permeabilities and gradients can be described by an extension of the basic Nernst equation:[50, 51]

$$E = -\frac{RT}{nF} \ln \frac{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o}{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}$$

If the permeability of one ion species is markedly greater than any other, the concentration gradient of that particular ion will predict the membrane potential. Following excitation of a nerve cell, the level of the membrane potential at any point in time during the depolarization and repolarization process will be a function of the relative permeabilities and gradients of the various ions.

Following return of the membrane to the resting potential level, a very slight excess of sodium ions is present within the cell and a very slight excess of potassium ions exists outside of the nerve cell. Although the excitation process has been completed and the nerve cell is electrically quiescent, a metabolically active period commences. Restoration of the normal ionic gradient across the nerve membrane requires the expenditure of energy for the active transport of sodium ions from the inside to the outside of the nerve cell against a concentration gradient. This active transport of sodium ions is made possible by the function of the so-called sodium pump. The energy required to drive the sodium pump is derived from the oxidative metabolism of adenosine triphosphate. It has been shown that dinitrophenol, which interferes with oxidative phosphorylation, can prevent the active transport of sodium from the nerve cell, which suggests that the sodium pump is dependent on phosphorylation mechanisms.[52] Addition of excess adenosine triphosphate to a dinitrophenol-treated axon can reverse the inhibition of sodium efflux and restore the rate of sodium extrusion to normal. This metabolic pump, which actively extrudes intracellular sodium ions, also is believed responsible, in part, for the transport of potassium ions from the extracellular space to the interior of the nerve cell, since potassium ions also must move against a concentration gradient in order to restore the normal K_i/K_o ratio across the cell membrane. Potassium will return to the interior of the cell until the electrostatic attraction of the intracellular negative charges balances the chemical concentration gradient.

MECHANISM OF ACTION OF LOCAL ANESTHETIC AGENTS

Electrophysiological Actions

On the basis of the electrophysiological properties of peripheral nerve, it is conceivable that local anesthetic agents could interfere with the excitation process in nerve membrane in one or more of the following ways: alteration of the basic resting potential of the nerve membrane; alteration of the threshold potential or firing level; decrease in the rate of depolarization; and prolongation of the rate of

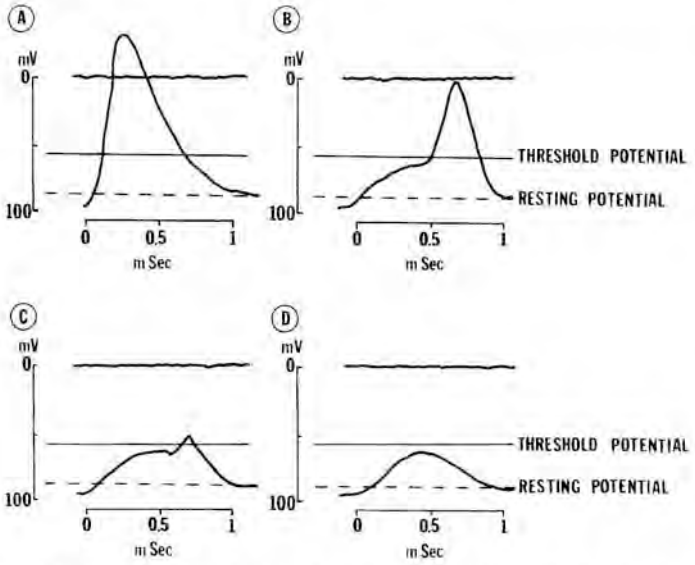


Fig. 2-9. Effect of 0.2 m*M* lidocaine on nerve membrane action potential. Panel A is a control recording, Panels B, C, and D represent recordings following exposure to lidocaine.

repolarization. Figure 2-9 shows the changes in the transmembrane action potential which occur following immersion of an isolated nerve in a local anesthetic solution. No alteration in the membrane resting potential of isolated nerves has been observed following exposure to varying concentrations of different local anesthetic agents such as procaine or lidocaine.[53,54] In addition, little or no change in the threshold potential or firing level occurs following application of local anesthetic agents to an isolated nerve. Thus, these compounds do not impede the excitation process in nerve by altering either the resting potential or threshold potential. The predominant electrophysiological alteration occurs during the depolarization phase of the action potential. Studies by Aceves and Machne have shown a decrease in the maximum rate of rise of the action potential of the isolated lumbar spinal ganglion of the frog from a control value of 190 v/sec to 120 v/sec after 15 minutes exposure to a solution of 0.005% (0.2 m*M*) lidocaine.[54] This marked decrease in the rate of the depolarization phase, particularly the phase of slow depolarization, is not accompanied by any significant change in the rate of repolarization. In summary, the primary electrophysiological effect of local anesthetic agents on the nerve membrane involves a reduction in the

rate of rise of the depolarization phase of the action potential. When cellular depolarization is not sufficient to reduce the membrane potential of the individual fiber to the firing, or threshold potential, a propagated action potential fails to develop.

Effect on Ionic Flux

Since the depolarization phase of the action potential is associated with an influx of sodium ions from the extracellular to intracellular space and since the primary electrophysiological effect of local anesthetic agents involves the depolarization phase of the action potential, it appears logical that local anesthetic agents probably interfere with sodium permeability. Condouris evaluated the interrelationship between sodium ions and cocaine on the surface action potential of the isolated frog sciatic-peroneal nerve trunk.[55] A series of dose-response curves relating concentration of cocaine and the height of the spike potential were determined in Ringer's solution containing various concentrations of sodium. As the concentration of sodium in the bathing solution was decreased, substantially less cocaine was required to reduce the height of the spike potential. As shown in Figure 2-10, at a normal sodium concentration of 116 mmoles, approximately 3.2 mmoles of cocaine were required to produce a 50% decrease in the height of the spike potential. When the sodium concentration was lowered to 12 mmoles, only 0.15 mmoles of cocaine

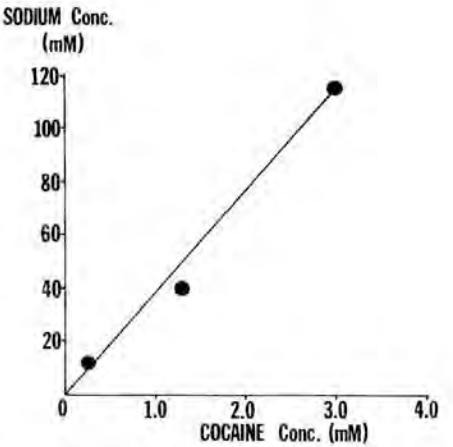


Fig. 2-10. Relationship between sodium and cocaine concentration required to produce a 50% decrease in nerve-surface action potential.

were necessary to cause a similar reduction in the amplitude of the spike potential. These data suggest that a competitive antagonism exists between local anesthetic agents and sodium with regard to depolarization of the nerve cell.

Direct measurements of sodium and potassium conductance have been carried out utilizing voltage clamp techniques[56-61] to demonstrate that local anesthetic agents block sodium currents in nerve. For example, Hille used the isolated frog sciatic nerve, in which the membrane potential was held at the normal resting potential of −75 mv and demonstrated that 1 mmole of lidocaine produced a complete loss of sodium current.[57] This effect was attributable to a reduction of sodium conductance by lidocaine. At the same time, 3.5 mmoles of lidocaine caused only a 5% decrease in potassium conductance. Therefore, although a decrease in permeability of the cell membrane to potassium can be observed when high local anesthetic concentrations are applied to isolated nerve, this reduction in potassium permeability is considerably less than the decrease in sodium permeability produced by significantly lower concentrations of the local anesthetic agents. These data agree with electrophysiological studies, which show primarily a decrease in the rate of depolarization following exposure of isolated nerves to local anesthetic drugs and only a slight prolongation of repolarization. Additional evidence that the reduction of potassium currents is not an essential component of conduction blockade by local anesthetic agents was obtained in studies in which tetraethylammonium and tetrodotoxin were utilized. Potassium conductance can be blocked completely by tetraethylammonium in frog-myelinated nerve fibers without any accompanying inhibition of the action potential.[62] On the other hand, tetrodotoxin, the puffer fish poison, causes complete inhibition of sodium conductance and complete blockade of the action potential of isolated nerves at a concentration of 30 n*M* without any discernible effect on potassium conductance.[57] These data indicate that the primary action of local anesthetic agents involves: (a) a reduction in the permeability of the cell membrane to sodium ions; (b) subsequent decrease in the rate of rise of the depolarization phase of the action potential; and (c) failure of a propagated action potential to develop, which ultimately causes conduction blockade.

Calcium-Local Anesthetic Interaction

Calcium ions, which are known to exist in the membrane in a bound state, may exert a regulatory role on the movement of sodium ions across the nerve membrane. The release of bound calcium has

been suggested as the primary factor responsible for the increase in the sodium permeability of the nerve membrane and, thus, may represent the initial step in the depolarization process.[63] Therefore, inhibition of sodium conductance by local anesthetic agents may be due, in part at least, to a local anesthetic-calcium interaction.[64-66] Electrophysiological studies by Aceves and Machne have examined the interrelationship of calcium and local anesthetic drugs.[54] Addition of procaine to the bathing solution surrounding an isolated nerve in the presence of a normal concentration of calcium produces a marked decrease in the rate of depolarization and the amplitude of the action potential and an inhibition of a propagated action potential. However, if the calcium concentration is increased from a normal value of 1.8 mmoles to 18 mmoles, the rate of depolarization and the height of the spike potential return to their control values despite the continued presence of the local anesthetic agent in the bathing solution, and ultimately a propagated action potential can be made to reappear. The reversal of the depressant effect of procaine on the rate of depolarization and amplitude of the isolated nerve action potential by an increase in the calcium concentration is demonstrated in Figure 2-11.

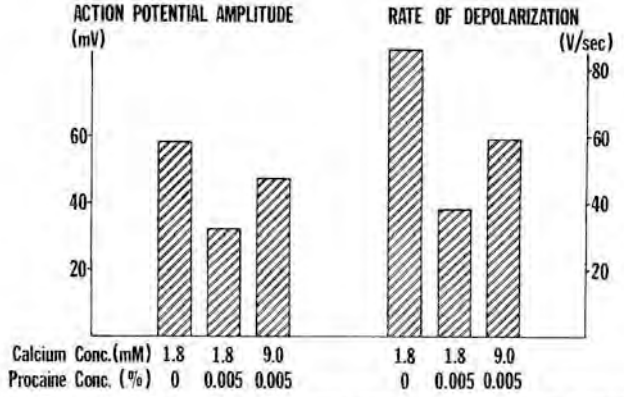


Fig. 2-11. Effect of calcium and procaine on action potential amplitude and rate of depolarization of isolated nerve.

In the absence of a local anesthetic agent, an increased calcium concentration alone produces inconsistent effects on the spike potential amplitude and actually decreases slightly the rate of depolarization.[54]

These results indicated that the local anesthetic molecules might compete with calcium for some site on the nerve membrane. Addi-

tional support for this hypothesis was forthcoming from the studies by Kuperman, Altura, and Chezar, in which isolated frog sciatic nerves were immersed in Ringer's solution containing labeled calcium (^{45}Ca) for 8 hours.[67] The nerves were then placed in calcium-free Ringer's solution and the rate of calcium efflux from nerve to the bathing solution was determined. Addition of 20 mmoles procaine to the nerve bath markedly accelerated the rate of efflux of radioactive calcium from the isolated nerve. A similar increase in calcium release by procaine was obtained in an isolated sartorius muscle preparation. In addition, a comparison of tetracaine and procaine revealed that 5 mmoles of tetracaine produces a 100% greater increase in rate of calcium efflux from sartorius muscle than 50 mmoles of procaine, which suggests a correlation between the anesthetic potency of procaine and tetracaine and their ability to displace calcium from the membrane.

Finally, an interrelationship has been demonstrated between calcium, local anesthetic agents, and sodium flux. Direct measurements of sodium conductance by voltage clamp techniques have shown that the local anesthetic inhibition of sodium conductance can be reversed by increasing the calcium ion concentration.[58, 68] Conversely, reduction in calcium concentration accentuates the inhibitory effect of local anesthetic drugs on sodium conductance. Moreover, saxitoxin binds specifically to the sodium channels in nerve membranes and calcium ions are capable of displacing saxitoxin from this binding site.[69]

Under normal physiological conditions, calcium is probably bound to the phospholipids which constitute the major portion of the lipid element in the cell membrane. Displacement of calcium from this phospholipid-binding site may represent the initial step in the increased permeability of the membrane to sodium ions that ultimately results in membrane depolarization. Blaustein and Goldman have studied the effect of various local anesthetic agents on the binding of calcium to an in vitro phospholipid model and have correlated this action with the local anesthetic activity of the compounds.[59] A reasonable correlation existed between the ability of these agents to suppress the surface action potential of the isolated frog sciatic nerve and their ability to inhibit calcium binding to phosphatidyl-L-serine (Fig. 2-12). For example, lidocaine was found to be 3.5 times as potent as procaine in terms of inhibiting the binding of calcium to phosphatidyl-L-serine and 3.8 times as potent as procaine in blocking conduction in the isolated frog sciatic nerve. Although the above studies clearly indicate an interaction between calcium and local anesthetics, most authorities doubt that calcium plays an essential role in the pro-

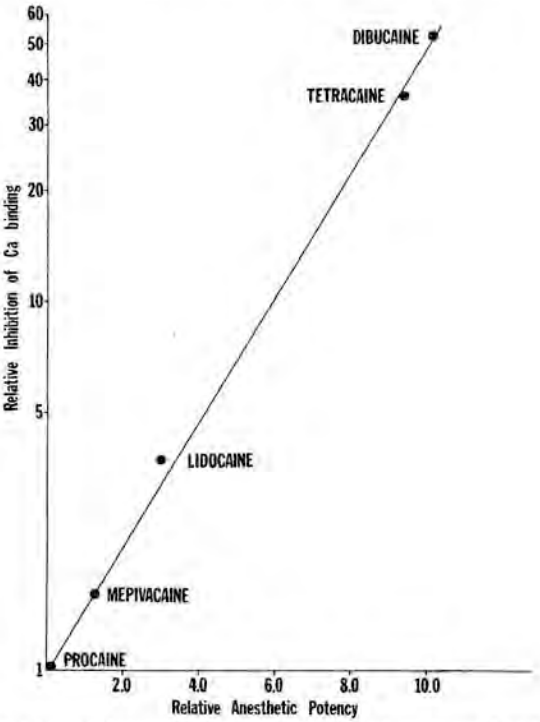


Fig. 2-12. Relationship of inhibition of calcium binding to anesthetic potency.

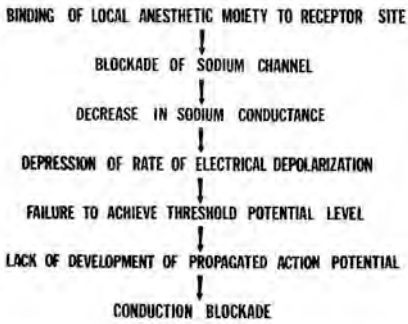
duction of local anesthesia. The following sequence is generally accepted as the mode of action of local anesthetic agents: a) reduction in permeability of the cell membrane to sodium ions; (b) decrease in the rate of depolarization of the membrane action potential; (c) lack of development of a propagated action potential; and (e) conduction blockade.

ACTIVE FORM OF LOCAL ANESTHETIC AGENTS

Most of the clinically useful local anesthetic preparations are available in the form of solutions of a salt. For example, lidocaine is usually prepared as an 0.5 to 2.0% aqueous solution of lidocaine hydrochloride. In solution, the salts of these local anesthetic compounds exist both in the form of uncharged molecules (B) and as positively charged cations (BH^+). The relative proportion between

Table 5

SEQUENCE OF EVENTS OF LOCAL ANESTHETIC BLOCK



the uncharged base (B) and the charged cation (BH^+) depends on the pH of the solution and on the pK_a of the specific chemical compound. The relation between these various factors can be expressed simply as follows:

$$pH = pK_a - \log (BH^+ - B)$$

The total concentration of the local anesthetic agent (C) in solution or at the site of injection is equal to the amount present in the cationic form and the amount present as base.

$$C = BH^+ + B$$

Since the pK_a is constant for any specific compound, the relative proportion of free base and charged cation in the local anesthetic solution depends essentially on the pH of the solution ($BH^+ \rightleftharpoons B + H^+$). Thus, knowledge of the pK_a of a specific compound, the pH of the solution, and the total concentration of the local anesthetic agent would make it possible to determine the relative amounts of the compound that are present in the cationic and base form. As the pH of the solution is decreased and the hydrogen ion concentration is increased, the equilibrium will shift toward the charged cationic form and, thus, relatively more cation will be present than free base. Conversely, as the pH is increased and hydrogen ion concentration decreased, the equilibrium will be shifted toward the free base form, and relatively more of the local anesthetic agent will exist in the free base form rather than as the charged cation.

Much of the early work carried out on isolated intact peripheral nerves indicated that local anesthetic agents were more efficacious when prepared as alkaline solutions. Since more of the uncharged base (B) is present in alkaline solutions, these results suggest that the uncharged base form of the local anesthetic moiety was responsible for the actual anesthetic activity.[70] However, these earlier studies probably failed to take into account that two factors are involved in the ultimate anesthetic action of a chemical compound, i.e., diffusion through the nerve sheath and binding at the receptor site in the cell membrane. To evaluate the difference between diffusion through the nerve sheath and binding at a receptor site, Ritchie, Ritchie, and Greengard carried out a series of studies evaluating the interrelation between the pH of solutions of lidocaine and dibucaine, presence or absence of the nerve sheath, and local anesthetic activity.[71, 72] These investigators used both an isolated frog sciatic nerve and an isolated rabbit vagus nerve preparation. When isolated nerves possessing an intact sheath were studied, it was found that as the pH of the bathing solution containing either lidocaine or dibucaine was raised from 7.2 to 9.2, the rate of reduction in the height of the surface action potential was markedly increased. Thus, alkaline solutions containing relatively greater amounts of the uncharged base (B) were more active in suppressing electrical activity of the sheathed nerve preparations. This observation was consistent with those previously reported by earlier investigators. However, when the experiment was repeated with the use of a desheathed frog sciatic nerve or rabbit vagus nerve preparation, the results differed. Under these experimental conditions, a less alkaline local anesthetic solution, which would result in the formation of a relatively greater amount of the charged cation (BH^+), increased local anesthetic activity. On the basis of these observations, Ritchie, Ritchie, and Greengard postulated that both the uncharged base form (B) and the charged cationic form (BH^+) of local anesthetic agents are involved in the total process of penetration and conduction block. The uncharged base form is believed responsible for optimal diffusion through the nerve sheath. After penetration of the sheath, re-equilibrium occurs between the base and cationic form, and, at the cell membrane itself, the charged cation binds to the receptor site and is ultimately responsible for suppression of the electrophysiological events observed in peripheral nerve[73] (Fig. 2-13). Additional evidence supporting the cationic form of local anesthetic agents as the active moiety was forthcoming from studies in which quaternary ammonium analogues of lidocaine were employed.[74, 75] These compounds, which can only exist in the form of charged cations and not as uncharged bases, were found to be as

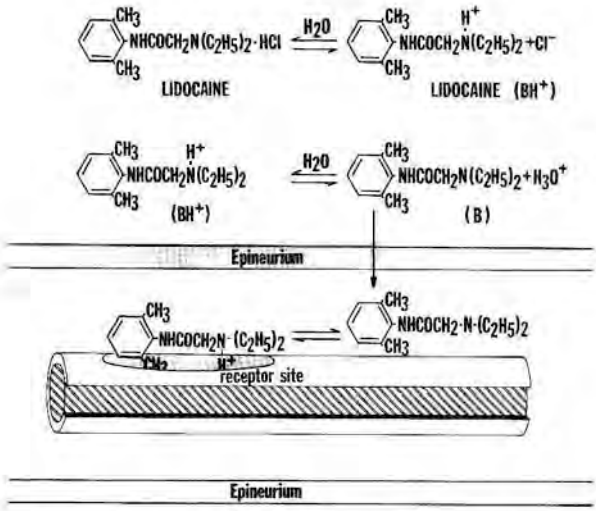


Fig. 2-13. Diffusion of base form of local anesthetic agent across epineurium and subsequent binding of cationic form with receptor site at nerve membrane.

active in blockade of conduction in peripheral nerves as their tertiary amine analogues. Furthermore, Catchlove has studied the permeability of isolated nerve sheaths to local anesthetic agents and has reported that the permeability of these agents is a linear function of the fraction of nonionized drug present in solution.[76]

Knowledge that the cationic form of local anesthetic agents is mainly responsible for the conduction blocking action of this class of compounds has definite clinical relevance. It has been known for many years that carbon dioxide can potentiate some of the actions of local anesthetic agents.[77] Catchlove has reported that CO_2 potentiation of local anesthetic activity is due, at least in part, to the increased formation of the active cationic form by lowering the pH at the nerve membrane where the local anesthetic receptor is believed to reside. Catchlove demonstrated that continuous equilibration of Ringer's solution containing local anesthetic agents with 9.6% carbon dioxide resulted in a significantly greater suppression of the height of the nerve action potential than achieved by either procaine, lidocaine, or bupivacaine without CO_2.[78] For example, 1 mmole bupivacaine reduced the height of the surface action potential of the isolated frog sciatic nerve to 62% of control value. Equilibration with 9.6% carbon dioxide and bupivacaine reduced the height of the surface action potential to 6% of the control value within 5 minutes. The role of the

Table 6

EFFECT OF CO_2 ON THE ANESTHETIC PROPERTIES OF LIDOCAINE AND PRILOCAINE IN EPIDURAL ANESTHESIA

	Onset Time Mean ± S.D.	Time to Complete Spread	Sensory Duration	Average Degree of Motor Block
2.0% LIDOCAINE HCl	5.5 ± 1.1	16.0±2.6	97±19.0	36.3%
1.75% LIDOCAINE CO_2	3.6 ± 0.9	10.6±1.6	108±12.8	51.5%
2.0% PRILOCAINE HCl	7.3 ± 1.8	17.3±2.4	97±10.5	36.3%
1.71% PRILOCAINE CO_2	4.1 ± 1.0	13.1±2.1	113±16.7	48.3%

cationic form of local anesthetic agents in this CO_2 potentiation was substantiated by the inability of carbon dioxide to potentiate the action of N-butanol, a nonionizable local anesthetic compound. Clinical studies have been conducted in which carbonated solutions of lidocaine and prilocaine have been employed for epidural anesthesia and brachial plexus blockade.[79–81] Bromage and co-workers have reported a significant decrease in the onset of epidural anesthesia and an improvement in the quality of epidural anesthesia with the carbonated solutions of lidocaine and prilocaine[79, 80] (Table 6). Similarly, a faster onset of sensory anesthesia following brachial plexus blockade has been reported when solutions of lidocaine equilibrated with CO_2 were employed.[81]

SITE OF ACTION OF LOCAL ANESTHETIC AGENTS

Electrophysiological and biochemical studies have clearly implicated the nerve membrane as the site at which local anesthetic agents exert their pharmacological action. Cellular metabolism of nerves is not inhibited by local anesthetic compounds employed in therapeutic concentrations.[82, 83] Although metabolic inhibitors are capable of producing conduction blockade, the nature of the block is considerably different from that caused by local anesthetic agents.[84] Adenosine triphosphate (ATP), which is intimately involved in reestablishment of the sodium-potassium gradient across the nerve membrane by way of the so-called sodium-potassium pump, has been reported to prevent and reverse conduction blockade by procaine in the isolated frog sciatic nerve.[85] This effect of ATP may be suggestive of a metabolic inhibitory effect of local anesthetic agents, which indirectly cause a decrease in sodium conductance. However, this antagonism between ATP and procaine was not shown to be competitive in nature, since

an increase in procaine concentration was not able to overcome the ATP antagonism. Moreover, conduction blockade due to an impairment of the sodium pump by an inhibitory effect on ATP should have been accompanied by a change in the membrane resting potential. As indicated previously, local anesthetic activity is not associated with a change in the resting membrane potential. Although the explanation for the ATP-procaine interaction is not clear, ATP inhibition is not believed responsible for the conduction blocking properties of local anesthetic drugs. In addition, these agents have been found to have no effect on the electrogenic component of the sodium pump in nerves.[86]

Membrane Receptor

The interaction between sodium ions, calcium ions, and local anesthetic agents is suggestive of a specific receptor site for local anesthetic agents at the nerve membrane. Two types of studies have been conducted to support the concept of a membrane receptor for local anesthetic agents. In vitro biochemical investigations have revealed that local anesthetic agents can bind to isolated proteins and phospholipids, which suggests that receptor binding sites for local anesthetic compounds could exist in the membrane.[87] Additional evidence supporting the presence of specific local anesthetic receptors is derived from studies in which optical isomers of local anesthetic agents have been separated and their relative ability to impede neural conduction evaluated.[88,89] Akerman has conducted a detailed investigation of the anesthetic properties of such optical isomers and has observed as much as a fivefold difference in intrinsic anesthetic potency between optical isomers of certain specific local anesthetic agents.[90, 91] These data have been interpreted as suggestive of specific steric requirements for the interaction of a local anesthetic drug and a receptor site in the excitable nerve membrane.

The exact location of a local anesthetic receptor in the nerve membrane also has been the subject of considerable investigation. Hille and co-workers[92] and Strichartz[93] have presented data which suggest that this local anesthetic receptor is probably located at or near the sodium channel in the nerve membrane. Futhermore, it has been postulated that receptors may be present either on the external surface of the sodium channel or on the internal axoplasmic surface of the sodium channel. Local anesthetic compounds have been classified according to their ability to react with receptor sites on either the external or internal portion of the sodium channel.

EXTERNAL RECEPTOR SITE

Only the two biotoxin substances, tetrodotoxin and saxitoxin, have been clearly demonstrated as inhibiting sodium conductance by an effect on the external surface of the sodium channel. Narahashi, Anderson, and Moore have perfused the giant squid axon both externally and internally with solutions of tetrodotoxin.[94] External perfusion with $1 \times 10^{-7}M$ tetrodotoxin prevented the development of a propagated action potential within 3–6 minutes. On the other hand, internal perfusion with $1 \times 10^{-6}M$ and $1 \times 10^{-5}M$ tetrodotoxin for 17–37 minutes had no effect on any component of the action potential. These data suggest that tetrodotoxin inhibits the movement of sodium ions by an interaction with receptor sites on the external surface of the nerve membrane.

INTERNAL RECEPTOR SITE

Certain quaternary ammonium compounds, structurally similar to clinically useful local anesthetic agents such as lidocaine have been found to block conduction when applied to the internal surface of the nerve membrane.[74] Application of these compounds to the external surface of the nerve membrane results in either no conduction blockade or an extremely slow development of block. For example, Strichartz has reported that sodium currents in single myelinated fibers obtained from the sciatic nerve of the frog were diminished only slightly by the external application of quaternary ammonium compounds related to lidocaine.[93] However, axoplasmic injection and infusion of these quaternary compounds inhibited sodium currents by more than 90%. On the basis of his studies, Strichartz has suggested that the receptor site for these quaternary ammonium compounds related to lidocaine is located halfway down the electrical gradient from the inside to the outside of the sodium channel. Hille, Courtney, and Dunn have presented evidence based on similar studies that the receptor site for tertiary amine local anesthetic drugs such as lidocaine is the same as that determined for the quaternary ammonium derivatives of these clinically useful local anesthetic agents.[92]

Membrane Reactive Sites

The evidence presented above has been interpreted by most investigators as suggestive of a receptor site for local anesthetic agents. However, alternative theories not involving a specific receptor have been proposed. Zipf has suggested that local anesthetic

agents do not necessarily bind with specific receptors on the cell membrane, but rather may interact with ubiquitous-type reactive sites.[95] The inhibition of conduction by local anesthetic agents then could be explained by a surface charge hypothesis.[96] This hypothesis suggests that the interaction of local anesthetic agents with these ubiquitous reactor sites results in the neutralization of the fixed negative charges in the cell membrane such that the potential across the membrane would rise although the recorded resting potential would remain constant. When this increase in the transmembrane potential is sufficiently great, electrotonic currents from neighboring unanesthetized nerve membranes would be insufficient to reduce the membrane potential to its threshold or firing level and conduction blockade would then occur.

Membrane Expansion

Although the cationic form of agents such as lidocaine, mepivacaine, prilocaine, and bupivacaine appears to be responsible for their local anesthetic action, other compounds are known to exist as uncharged molecules at physiological pH and still exert clinically useful anesthetic activity. Benzocaine, for example, does not exist in the cationic form and yet exhibits potent topical anesthetic properties. Studies by Ritchie and Ritchie on the relationship of pH to the conduction blocking properties of benzocaine have revealed that the anesthetic activity of benzocaine is unaffected by changes in pH.[97] It is difficult to conceive that such uncharged molecules could act at a specific receptor site in the same fashion as the cationic form of the conventional local anesthetic agents.

The membrane expansion theory, which actually preceded the specific receptor theory, has been reproposed as a possible explanation for the local anesthetic activity of compounds such as benzocaine. The original form of this theory postulated an increased lateral pressure in the membrane by local anesthetic agents, which could produce a constriction of the membrane channels through which sodium ions move.[70] In vitro studies by Skou, in which the surface pressure of monomolecular layers of lipids increased following placement of local anesthetic drugs into the solution below the lipid layers, served as the basis for this form of the membrane expansion theory.[98–100] More recently, Johnson and Murphy have suggested that an increased movement of lipid molecules produces a confirmational change in proteins associated with lipids in the cell membrane resulting in an expansion of the membrane.[101] Support for

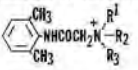
this view of the membrane expansion theory was presented by Seeman, who observed an inverse correlation between the membrane-buffer partition coefficient and membrane concentration required for conduction blockade of agents which exist mainly in the uncharged form.[102] These data have been interpreted as indicating that compounds which are highly lipid soluble can penetrate the lipid portion of the cell membrane more readily, causing a confirmational change in the lipoprotein matrix of the membrane, with a resultant decrease in the diameter of the sodium channels, which thereby results in an inhibition of sodium conductance and neural excitation.

Classification of Local Anesthetic Agents by Site of Action

Takman has proposed a biological classification of local anesthetic agents based on information currently available regarding site of action of local anesthetic drugs and the active form of these compounds[103] (Table 7). According to this classification, local anesthetic compounds can be categorized as follows:

Table 7

CLASSIFICATION OF LOCAL ANESTHETIC SUBSTANCES ACCORDING TO BIOLOGICAL SITE AND MODE OF ACTION

Classification	Definition	Chemical Substances
Class A	Agents acting at receptor site on external surface of nerve membrane.	Tetrodotoxin Saxitoxin
Class B	Agents acting at receptor site on internal surface of nerve membrane.	Quaternary ammonium analogues of lidocaine, e.g. 
Class C	Agents acting by a receptor independent physicochemical mechanism.	Benzocaine N-butanol Benzylalcohol
Class D	Agents acting by a combination of a receptor and receptor independent mechanism.	Most clinically useful local anesthetic agents, e.g. lidocaine mepivacaine prilocaine

Class A. Agents acting at a receptor site on the *external* surface of the sodium channel: tetrodotoxin, saxitoxin.

Class B. Agents acting at a receptor at the *internal* axoplasmic opening of the sodium channel: quaternary ammonium compounds, e.g., QX 314, QX 572, QX 222.

Class C. Agents acting by way of a receptor independent mechanism: benzocaine, N-butanol, benzylalcohol.

Class D. Agents acting both via a receptor mechanism and a receptor independent mechanism, i.e., procaine, lidocaine, mepivacaine, prilocaine, bupivacaine, and etidocaine.

Class A and B contain compounds that exist only in a charged form such as the biotoxins and quaternary ammonium analogues of lidocaine. The compounds in Class C are chemical substances which exist only in an uncharged form, such as benzocaine. The agents in Class D are anesthetic drugs that can exist in both a charged and uncharged form. Most of the clinically useful local anesthetic agents are in the last category. This classification implies that agents such as lidocaine and mepivacaine would exert their local anesthetic activity both by way of a cationic-receptor site interaction and by a base physicochemical disturbance within the nerve membrane. On the basis of studies carried out on the squid giant axon, Narahashi and Frazier suggested that approximately 90% of the blocking effects of agents such as lidocaine was due to the cationic form of the drug and approximately 10% of the blocking action was caused by the base form.[104]

SUMMARY

1. Peripheral nerves are enclosed in connective tissue sheaths, the endoneurium, perineurium, and epineurium, which act as barriers through which local anesthetic agents must diffuse. In addition, the presence of a myelin sheath in some nerve fibers and a cell membrane, which are basically lipoprotein in nature, also affects the action of anesthetic drugs.
2. Neural excitation is associated with a depolarization and repolarization of the cell membrane. The depolarization phase results from an increased membrane permeability to sodium ions, whereas the main determinant of the repolarization phase is increased potassium conductance.
3. Local anesthetic agents inhibit neural excitation by impeding sodium conductance, which thereby prevents membrane depolarization.
4. Most of the clinically useful local anesthetic drugs probably act by displacement of calcium from a lipoprotein receptor site lo-

cated on the internal surface of the cell membrane. This anesthetic-receptor interaction results in blockade of the membrane sodium channel which, in turn, decreases sodium permeability and inhibits membrane depolarization.

5. Most of the clinically useful local anesthetic agents exist in both the charged and uncharged (base) form in solution. The uncharged base form diffuses more readily through neural sheaths, while the charged form is mainly responsible for attachment to the membrane receptor and ultimate blockade of neural activity.
6. The relative proportion of charged and uncharged form is dependent upon the pK_a of the chemical substance, pH of the anesthetic solution, and pH at the injection site. These factors will affect the pharmacological profile of different agents such as onset time, anesthetic potency, and duration of action.