Local Anesthetics: Significance of Hydrogen Bonding in Mechanism of Action

Abstract. *The action of local anesthetics is considered in terms of their ability to function as the donor in a hydrogen bond. The formation of a hydrogen-bonded complex between the drug and an acceptor group on the neural membrane is suggested as a feature in the action of local anesthetics.*

It has been suggested that local anesthetics stabilize the membrane potential by combining chemically with polar groups of either the lipoprotein (*1*) or the phospholipid (*2–4*) in the neural membrane. Although several mechanisms have been advanced to explain local anesthetic action (*2–7*), none consider that the drug may be bound to the membrane by participating as a hydrogen bond donor. We report data that support the idea that local anesthetics form a hydrogen-bonded complex with a receptor in the membrane. All of the compounds commonly designated and used as local anesthetics possess, at physiological *p*H, at least one polar group capable of participating as the donor in a hydrogen bond.

From studies in model systems (*2–4*) it was observed that local anesthetics interfere significantly with the binding of calcium ions by phospholipids. It was further demonstrated that phosphodiesters are precipitated by local anesthetics. This affinity toward the phosphodiester group was demonstrated with a variety of compounds in addition to phospholipids such as polynucleotides, phosphoproteins, and simple organic phosphodiesters. On the basis of these findings it was suggested that local anesthetics would inhibit nerve conduction by replacing calcium ions in the formation of a more stable electrostatic complex with phospholipids in the neural membrane.

The nature of the interaction in a complex between a phosphodiester group and a local anesthetic is seen in the results of the crystal structure analyses of procaine bis-*p*-nitrophenyl phosphate (*8*) (Fig. 1) and phenacaine bis-*p*-nitrophenyl phosphate monohydrate (*9*) (Fig. 2). Both structures were determined from three-dimensional, x-ray diffraction data. In both of the crystalline complexes the local anesthetic is in the cationic form. The proton adds to the tertiary amino moiety in procaine and to the imino nitrogen in phenacaine. The molecular dimensions of the procaine molecule show that the *p*-aminobenzoate group has a small amount of quinoid character which imparts a fractional positive charge to the *p*-amino nitrogen atom, as predicted by Feinstein (*2*). Furthermore, he assumes in proposing a model for a complex between phosphatidyl serine and tetracaine that both positive nitrogens of the drug molecule are oriented toward negative oxygen atoms in adjacent phosphodiester groups. This feature agrees with the observed structure in the crystalline procaine complex where the amino and phosphodiester groups are contiguous. Suggested relative orientations for the local anesthetics and the phospholipids in the membrane (*2, 4*) are in accord with the molecular packing arrangement in the procaine crystal. While the suggested orientations were derived by considering only the electrostatic interaction between positive nitrogen and negative oxygen atoms, the solid state structures show that hydrogen bonding is an important additional aspect of the interaction. All of the amino hydrogen atoms in both crystal structures are utilized for hydrogen bonding. In the procaine complex, the hydrogen bond receptors are exclusively phosphodiester oxygen atoms. Consequently, the oppositely charged groups are in close contact as predicted. However, the situation differs somewhat in the structure of phenacaine bis-*p*-nitrophenyl phosphate monohydrate. In this case only one of the nitrogen atoms is directly hydrogen-bonded to a phosphodiester group even though there is a formal charge of plus one-half on each as a result of resonance in the amidinium ion. A water molecule is interposed between the other nitrogen and a phosphate oxygen atom. Nevertheless, hydrogen bonds join the water molecule to this nitrogen and to the phosphate group. If phospholipids are the membrane binding sites, then these results show that the local

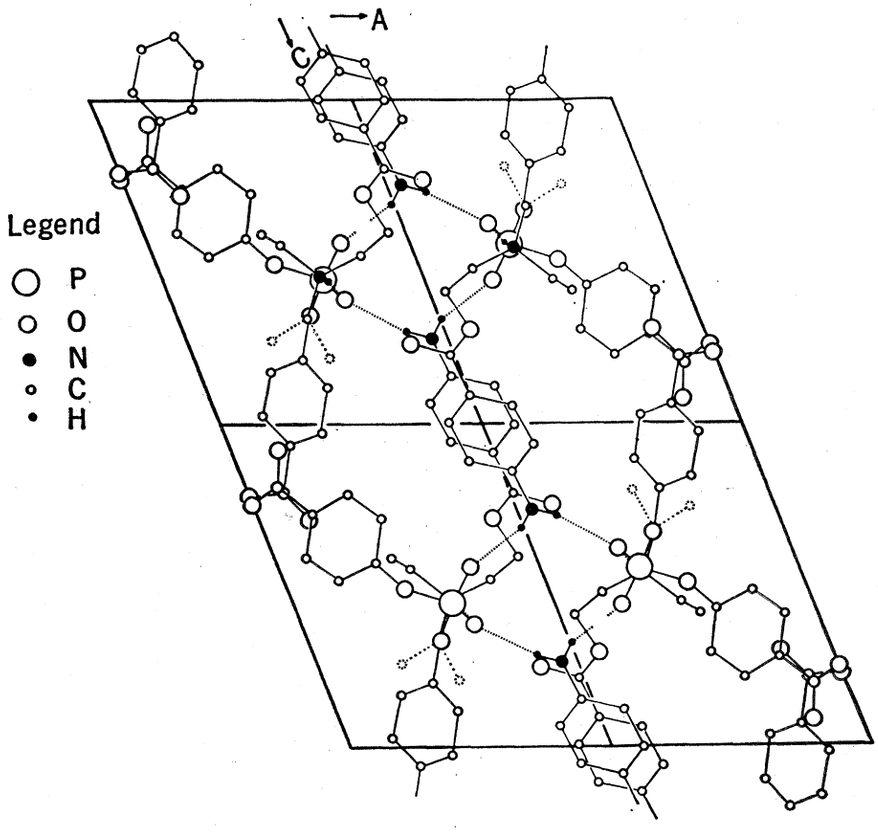

Fig. 1. Projection down the *b*-axis of a unit cell of procaine bis-*p*-nitrophenyl phosphate. Hydrogen bonds are indicated by dotted lines. A disordered terminal methyl group in the procaine molecule is also indicated by a dotted line. Only the amine nitrogens (those involved in hydrogen bonding) and not the nitro nitrogens are indicated by the solid circles.

anesthetics are held at least partially by hydrogen bonds. Furthermore, water bound to the membrane may also have a role in these complexes.

Phospholipids have been suggested as possible binding sites for local anesthetics because of their observed interaction with both calcium ions and anesthetics and because of the correlation between drug potency and its lipid solubility (*4*). Nevertheless, the results of crystal structure analyses indicate that any chemical group on the lipoprotein membrane which can accept a hydrogen bond is also a potential binding site for local anesthetics. One such plausible site of action for local anesthetics is the acetylcholine receptor. Although the chemical identity of this site is yet unknown, it is reasonable to expect it to contain a negative group (a potential hydrogen bond acceptor) (*10*). As is evident from its stereochemistry (*11*), acetylcholine could act as the acceptor in a hydrogen bond to a group in the receptor site, but it cannot function as the donor. Local anesthetics can participate in both capacities. It seems plausible that the additional hydrogen bond energy could stabilize a local anesthetic-receptor complex relative to one containing acetylcholine. In this way the latter compound would be blocked from the active site and hence transmission would be inhibited. Molecules closely related chemically and sterically to acetylcholine block electrical activity along the axon as well as at the synaptic junction (*6, 12*) in experiments supporting the idea that acetylcholine mediates axonal conduction (*13*). Among those compounds tested were derivatives of benzoylcholine and their tertiary analogs (*12*). Although the latter bear less of a structural resemblance to acetylcholine than the former, in every instance they blocked axonal conduction more effectively than the former. As these experiments were performed at *p*H 7.8, it seems reasonable to attribute the greater potency of the tertiary compounds to the formation of a more stable hydrogen-bonded complex with the receptor site as well as to the greater lipid solubility of the un-ionized species. It should be noted that benzoylcholines are not hydrogen bond donors.

Whether the ionic and nonionic local anesthetics act by a single mechanism has been widely discussed. Experiments on the cooperative influence of benzyl alcohol and procaine on blocking electrical activity in the axon show that these drugs have a single mechanism of action (*14*). Significantly, both are hydrogen bond donors. According to our interpretation these compounds hydrogen bond to a common acceptor on the membrane. Molecules that are not hydrogen bond donors may possess some of the local anesthetic property, but if the strong bond with the membrane acceptor is lacking, the anesthesia will be of short duration and the potency of the drug will be low (*15, 16*).

Table 1. Comparison of effectiveness of several local anesthetics. Data obtained from the initial slope of the curves obtained from Ritchie and Ritchie (*17*) of the relative decrease of the C elevation of the compound action potential with time. The calculated values of the effectiveness (RE) per millimole of anesthetic per minute were normalized with respect to procaine at *p*H 7.2.

Compound	Form	*p*H	RE
Procaine	Cationic	7.2	1.0
	Neutral	9.2	2.0
Benzocaine	Neutral	7.2, 9.2	1.5
Lidocaine	Cationic	7.2	5.4
	Neutral	9.2	1.0
Dibucaine	Cationic	7.2	17.7
	Neutral	9.2	3.2

The experimental observations of Richie and Richie (*17*) on the effect of *p*H on the activity of local anesthetics are consistent with the formation of a hydrogen-bonded complex between membrane and anesthetic. They tested the effectiveness of dibucaine, lidocaine, procaine, and benzocaine at *p*H 7.2 and 9.2. Although a difference in effectiveness between the cationic and the neutral form of a particular molecule was demonstrated, the differences were no greater than those between different molecules. From this data (*17*) an estimate of the effectiveness per millimole per minute was obtained (Table 1). The values compare favorably with the results of Blaustein and Goldman (*4*) on the relative inhibition of the binding of calcium ions to phosphatidyl-L-serine by local anesthetic reagents and with the relative anesthetic potency of the compounds at *p*H 7.2. Since the molecules are potential hydrogen bond donors under the conditions tested, hydrogen bonding could account for the primary polar interactions. The observed differences in activity between different molecules and different forms of the same molecule quite likely are the result of changes in both physicochemical and structural properties. The ability to participate in nonpolar interactions is a significant factor in considering the action of local anesthetics. Indeed, this feature may well explain the anomalous behavior of procaine in which the neutral form is more active than the cationic one. In the cationic form both ends of the molecule are polar and capable of serving as a hydrogen bond donor. In the neutral form, the *N,N*-diethylaminoethyl ester group becomes considerably less polar, permitting increased lipophilic interaction, while the aminobenzoate end remains available as a hydrogen bond donor.

In summary, the three-dimensional structures of the complexes between the local anesthetics procaine and

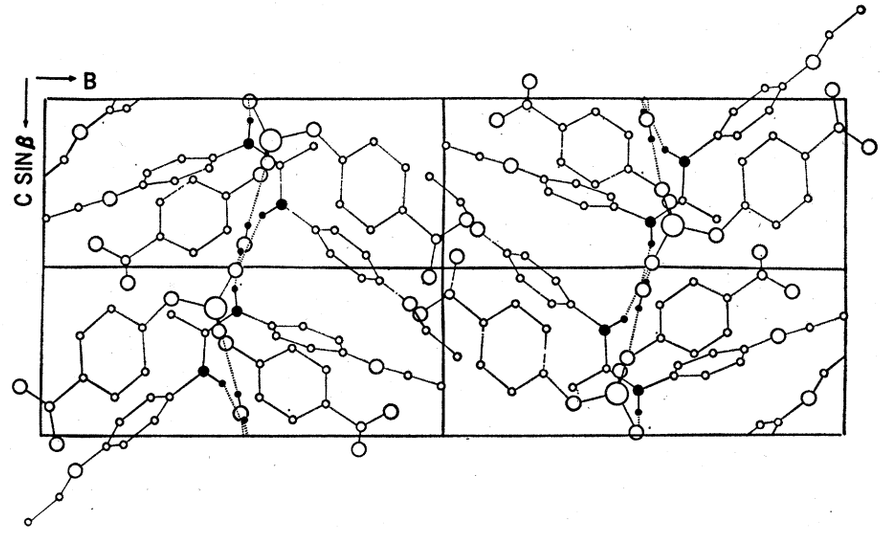

Fig. 2. Projection down the *a*-axis of a unit cell of phenacaine bis-*p*-nitrophenyl phosphate monohydrate. Legend in Fig. 1 also applies to this figure. Hydrogen bonds are indicated by dotted lines.

phenacaine and the phosphodiesters show the importance of hydrogen bonding in the interaction between the constituents of the complex. The formation of a hydrogen-bonded complex is a feature common to the functioning of useful local anesthetics, although apparently it is not obligatory that a drug be a potential hydrogen bond donor in order to block nerve conduction. Furthermore, the formation of a hydrogen-bonded complex as a significant feature in the mode of action of local anesthetics is compatible with several current theories of nerve conduction.

M. SAX
J. PLETCHER
Biocrystallography Laboratory,
Veterans Administration Hospital,
Pittsburgh, Pennsylvania 15240

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15. Local infiltration therapy with local anesthetics has been used for a long time in the management of pain. Relief frequently persists for periods ranging from hours to months after the block has worn off [John J. Bonica, *The Management of Pain* (Lea & Febiger, Philadelphia, 1953), chap. 13, p. 540]. An explanation for the duration of the relief is provided by our mechanism. The strong hydrogen-bonded complex with a membrane acceptor results in a slow rate of removal of the local anesthetic from its binding site. Consequently, the drug can remain for a considerable time at concentrations that are too small to block nerve conduction completely but are sufficient to increase the stability of the polarized membrane significantly.
16. Propanidid, {4-[(diethylcarbamoy)methoxy]-3-methoxyphenyl} acetic acid propyl ester, is an experimental short-acting intravenous anesthetic. This compound has the aromatic rings and side chains normally present in local anesthetics, but the diethylamide group is not a hydrogen bond donor at physiological *p*H.
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Nalorphine: Increased Sensitivity of Monkeys Formerly Dependent on Morphine

Abstract. *Three rhesus monkeys* Macaca mulatta, *formerly dependent on morphine, had increased sensitivity to nalorphine's effect of suppressing operant responding for food, as compared with two monkeys with no history of morphine exposure. Within the dose range employed, nalorphine injections produced emesis, salivation, and hyperirritability in formerly morphine-dependent monkeys but not in controls.*

An injection of nalorphine, a potent antagonist of morphine, immediately elicits an abstinence syndrome in morphine-dependent organisms which resembles, in nearly all aspects, the syndrome associated with the abrupt withdrawal of morphine. Because low doses of nalorphine have few effects on nondependent organisms, this drug is used extensively to determine the presence and magnitude of opioid physical dependence. A period of chronic exposure to morphine in man, or exposure to a single dose of morphine in rats, results in a decreased sensitivity (tolerance) to certain of morphine's effects which persist for several months (*1*). However, the possibility that organisms formerly dependent on morphine might show significant changes in sensitivity to drugs other than morphine has not yet been explored. We here demonstrate that rhesus monkeys formerly dependent on morphine have an increased sensitivity to nalorphine that appears to be the result of long-lasting physiological changes developed during periods of morphine dependence.

Prior to this experiment three monkeys were maintained for 2 months on 12 mg of morphine sulfate per kilogram (body weight) per day, given as

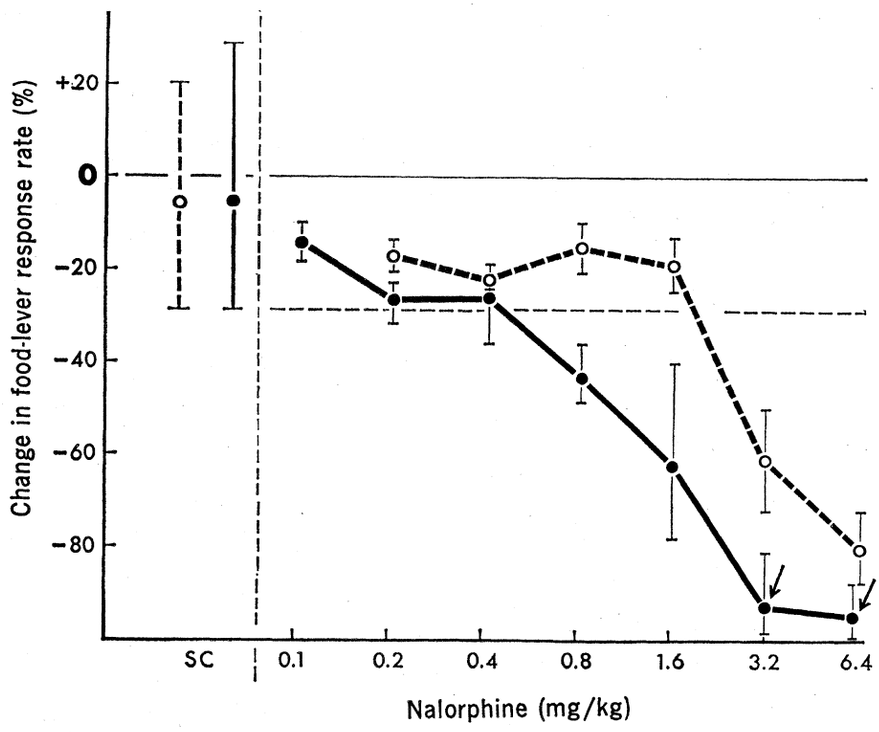

Fig. 1. Average percentage change in rate of food-lever response from the 5-minute period preceding the intravenous injection of saline or nalorphine to the 5-minute period after the injection of saline or nalorphine. The two points at the far left represent the average and the brackets represent the range of four saline control (*SC*) sessions. The horizontal dotted line indicates the lower limit of the range on saline control sessions. At each of the doses of nalorphine the points represent the average percent change in rate of food-lever response and the brackets represent the range. Arrows indicate the observation of emesis, excessive salivation, and hyperirritability. (Open circles) Control monkeys (no history of dependence on morphine), $N = 2$; (solid circles) monkeys withdrawn for 3 months from morphine dependence, $N = 3$.