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Cationic Amphiphilic Drugs and Phospholipid Storage Disorder*

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I. Introduction

A. What Is Drug-induced Phospholipidosis?

Phospholipidosis (phospholipid storage disorder) refers to an excessive accumulation of phospholipids in the tissues. Phospholipids are essential structural components of animal cell membranes and cytoskeletons (Stryer, 1988). Consequently, their synthesis, utilization, and turnover are regulated in the cell. Drugs, chemicals, endogenous substances such as hormones, cofactors, and other agents may perturb this regulation resulting in phospholipidosis. Direct interactions of drugs and other xenobiotics with phospholipids, or indirect effects brought about through changes in the synthesis and metabolism of phospholipids, can lead to several significant alterations in cellular structure and function. The participation of various lipids in the regulation of cellular functions through inositol phosphatide second messengers and through hormones of the arachidonic acid cascade is being increasingly recognized (Champe and Harvey, 1988; Farese, 1988). Phospholipidosis may occur as the result of metabolic dysfunction and genetic disorders such as Niemann-Pick and Tay-Sachs disease (Terry and Weiss, 1963; Lazarus et al., 1967) or may occur due to long-term treatment with CADs‡ (Lullmann

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‡ Abbreviations: CAD, cationic amphiphilic drug; ATPase, adenosine triphosphatase.

et al., 1978; Hostetler and Matsuzawa, 1981; Camus, 1989; Joshi and Mehendale, 1989). Drug-induced excessive storage of phospholipids can occur in lung, liver, brain, kidney, cornea, adipose and several other tissues (De la Iglesia et al., 1974; Leech et al., 1984; Hostetler et al., 1985; Kacew, 1985; Martin and Standing, 1988; Pirovino et al., 1988). Excessive phospholipid storage has been shown to occur in a variety of animal species and in humans. Considerable species variation exists with regard to the degree of phospholipidosis that occurs and the target organ being affected (Schmien et al., 1974; Sakuragawa et al., 1977; Lullmann-Rauch, 1979; Reasor, 1981; Miles et al., 1986; Kacew, 1987; Martin and Standing, 1988; Kodavanti and Mehendale, 1991).

The time required for development of phospholipidosis varies depending on the dose, type of drug, animal species, or the nature and origin of the cells used in the cell culture system. The use of cultured isolated macrophages and bovine pulmonary artery endothelial cells in studying the mechanism of phospholipidosis and other effects of CADs has become increasingly popular (Ruben et al., 1985; Martin et al., 1989). This approach has the special advantages of avoiding extensive metabolism of CADs and having a relatively short time required for the development of changes in phospholipid metabolism (Ruben et al., 1985). Several morphological, biochemical, and functional changes accompany phospholipidosis. These include an increase in cellular phospholipids, the appearance of lamellated inclusion bodies (lamellar bodies) in the cells, and macrophage infiltration, particularly in the lung (Reasor, 1981; Fernandez et al., 1986; Kodavanti and Mehendale, 1991). Excellent reviews of many of these aspects have appeared in the literature (Shikata et al., 1972; Lullmann-Rauch and Scheid, 1975; Lullmann et al., 1975; Michell et al., 1976; Lullmann-Rauch, 1979; Reasor, 1981, 1989; Hruban, 1984; Kacew, 1984; Kacew and Reasor, 1985; Martin and Rosenow, 1988a,b). Some of the reviews were published many years ago and in some relatively narrow aspects of phospholipidosis were examined. Furthermore, more rapid and new developments in this area make the availability of a more detailed and up-to-date review of phospholipidosis timely and desirable.

We have attempted to cover as many diverse aspects of drug-induced phospholipidosis as possible including characteristics, consequences, influence of drugs, relation to metabolism and disposition of drugs, species variation, and tissue specificity, which may shed some light on the mechanism of drug-induced phospholipidosis. Because one of the remarkable features of drug-induced phospholipidosis is infiltration of alveolar macrophages, an account of how macrophages play an important role in the etiology of pulmonary phospholipidosis also is detailed in this review. The mechanism of action of CADs with relation to phospholipidosis and the phospholipid-signaling system is given special consideration.

B. Pharmacology and Therapeutic Uses of Drugs Known to Induce Phospholipidosis

In this review we focus primarily on phospholipidosis induced by CADs, because this group of drugs is by far the most well studied in this regard. CADs, having diverse therapeutic uses (fig. 1), are known to induce phospholipidosis and alter phospholipid metabolism to a variable extent. Extensive use of these drugs and clinical evidence of the side effects associated with their use have necessitated increased investigation of drug-induced phospholipidosis. Drugs with phospholipidosis-inducing propensity include examples from almost every class of pharmacological agents including antipsychotics, antidepressants, antiarrhythmics, antianginals, antibacterials, antimalarials, anorexic agents, cholesterol-reducing agents, etc. (Lullmann-Rauch and Scheid, 1975; Joshi et al., 1988; Joshi and Mehendale, 1989; Reasor, 1989).

In contrast to their diverse pharmacological actions and therapeutic applications, these drugs share several common physicochemical similarities. The most predominant physicochemical properties shared by CADs are a hydrophobic ring structure on the molecule and a hydrophilic side chain with a charged cationic amine group (fig. 1), which impart the characteristic amphiphilicity inherent in these drugs. Therefore, these classes of drugs are termed cationic amphiphilic amines. Hydrophobic characteristics allow the molecules to permeate through the plasma membrane when they are not ionized. The ionized form usually remains associated with the membrane and induces membrane structure perturbation (Seydel and Wassermann, 1976; Lullmann et al., 1978). CADs interact with negatively charged and neutral polar lipids, such as phospholipids. The membrane phospholipids and their charged ionic groups regulate CAD entry and binding in the cells.

Therapeutic actions of many anesthetics, β-blockers, antipsychotics, antiarrhythmics, etc. are mainly elicited by their actions on ion channels and receptors (Levy and Richards, 1966; Luxnatt and Galla, 1986; Zychlinski and Montgomery, 1986; Nagai et al., 1987). The antiarrhythmic drug, amiodarone, blocks the entry of Na^+ in to the cell and also modulates cellular Ca^{2+} homeostasis (Mason et al., 1984; Chatelain et al., 1985). Whether these actions of amiodarone are manifested through changes in membrane composition, decreased transition temperature, or membrane fluidization or are receptor mediated remains to be investigated. Another antiarrhythmic drug, propranolol, is a potent β-adrenergic blocker and its action is receptor mediated (Levy and Richards, 1966; Patil, 1968). Antipsychotic drugs and other neuroleptics, such as chlorpromazine, promazine, and imipramine (fig. 1), are highly lipid soluble and surface active. Very high octanol to water partition coefficients of these drugs are indicative of a high solubility in biological membranes (Seeman, 1972; Seydel and Wassermann, 1976; Lullmann and Wehling, 1979; Welti et

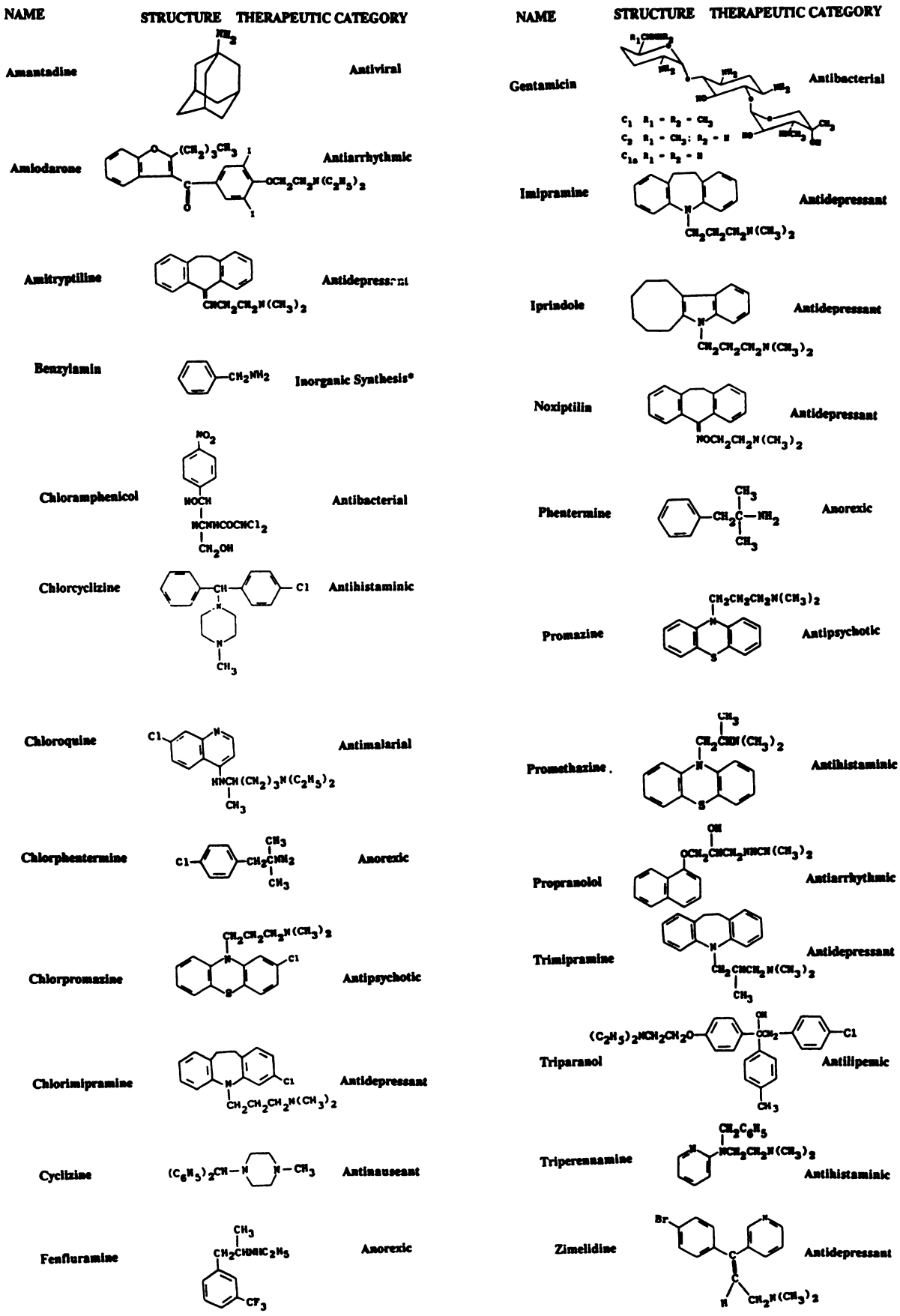

FIG. 1. Structures and therapeutic categories of CADs. *, not a therapeutic agent.

al., 1984). At higher concentrations (mM range), these drugs interact with the membranes in a nonspecific way and induce fluidization of all presynaptic and postsynaptic membranes. These actions lead to enhanced spontaneous release of neurotransmitters and altered physiological responses (Seeman, 1977).

Membrane effects of CADs on phospholipids have been extensively studied (Seydel and Wassermann, 1976; Lullmann and Wehling, 1979; Phadke et al., 1981; Brasseur et al., 1985; Henry et al., 1985; Chatelain et al., 1986; Joshi et al., 1989). There is a good correlation between the interaction of these drugs with phospholipids and their phospholipidosis-inducing capacity in vivo (Joshi and Mehendale, 1989; Joshi et al., 1989). However, it is not clear whether drug-phospholipid physicochemical interactions make phospholipids unsuitable substrates for the action of phospholipases or whether drugs directly inhibit the action of phospholipases by binding to the active site. There is some evidence for both of these mechanisms, and it is not inconceivable that a given drug may cause phospholipidosis through both of these mechanisms (Lullmann and Wehling, 1979; Kubo and Hostetler, 1985; Joshi et al., 1988, 1989). Detailed mechanistic considerations will be discussed later in the appropriate sections.

C. Characteristics of Phospholipidosis

Phospholipidosis usually appears after chronic intake of CADs. The latency for appearance of phospholipidosis varies from a few days to months, depending on animal species (Yamamoto et al., 1971a,b; Seiler and Wassermann, 1975; Lullmann et al., 1978; Blohm, 1979; Hostetler and Matsuzawa, 1981; Reasor, 1981). In isolated cultured cells, lamellar bodies filled with phospholipids can appear within a few hours of exposure to CADs.

There are three major changes that occur in lung tissue of animals receiving CADs that are capable of inducing phospholipidosis. One hallmark of pulmonary phospholipidosis is the infiltration of alveolar macrophages and other lymphocytes in the alveolar spaces. In the other tissues such as liver and kidney, macrophage infiltration has not been characterized. A detailed account of the role of alveolar macrophages in drug-induced pulmonary phospholipidosis is given in the following section. Irrespective of the type of tissue being affected, phospholipidosis is usually evident in the form of membranous lamellar bodies in the cells (Reasor, 1989). Normally, lamellar bodies are present in type II cells and surfactant and in the alveolar macrophages (Fisher and Chander, 1985), signifying their role in synthesis, secretion, and turnover of alveolar surfactant. These lamellar bodies increase in number and enlarge in size during CAD treatment (Hruban et al., 1972; Drenckhahn et al., 1976; Kannan et al., 1989). Although lamellar bodies have been thought to originate from lysosomes (Reasor, 1989), the origin and function of this lysosomal involvement remains all but uncertain. It has been proposed that lysosomes take up newly synthesized surfactant by an autophagic mechanism and process it to the characteristic lamellar bodies before it is released from the cell by exocytosis (Heath and Jacobson, 1976, 1980a,b; Notter and Finkelstein, 1984; Rooney, 1984, 1985; Fisher and Chander, 1985; Dobbs, 1989; Tierney, 1989; Wright and Hawgood, 1989). Lung cells, particularly type II cells and alveolar macrophages, are involved in the synthesis and recycling of alveolar surfactant. Many hydrolytic enzymes, including phospholipases, also have been detected in lamellar bodies of the lung (DiAugustine, 1974). The contents of lamellar bodies are secreted into the alveolar spaces by exocytosis. Secreted inclusion bodies act as newly synthesized surfactant material in intraalveolar linings (Wright and Hawgood, 1989). This material is recycled by the pinocytic action of alveolar macrophages and by reuptake into type II cells via endocytosis (Van Golde, 1985; Stern et al., 1986). Recaptured surfactant is digested by lysosomal phospholipases leading to the formation of precursors for new surfactant synthesis (Rooney, 1984, 1985). Surfactant material is known to contain higher numbers of lamellar inclusion bodies after CAD treatment (Hruban, 1984; Miles et al., 1986; Israel-Biet et al., 1987).

Either one or more of phospholipid-recycling and metabolism processes, which occur in lamellar bodies and lysosomes, are affected by CADs that induce phospholipidosis. A detailed account of how phospholipases and other enzyme systems are affected by CADs leading to the development of phospholipidosis appears in "Mechanism of Phospholipidosis".

The understanding of drug accumulation in lamellated bodies, their interaction with phospholipids as well as phospholipases, changes in the intraorganelle pH, and its relation to the pathobiology of these structures might help to further define the relationship of drug-induced phospholipidosis and the increased appearance of lamellar bodies.

CADs have several other side effects in addition to inducing phospholipidosis (Hobbs et al., 1959; Whisnant et al., 1963; Lewis et al., 1983; Manolis et al., 1987; Mason, 1987; Stein et al., 1987; Dunn and Glassroth, 1989; Vrobel et al., 1989; Young and Mehendale, 1989). The direct relationship between phospholipidosis and other side effects is not established, because these side effects are specific for a drug and not a general manifestation of all CADs. One example of uncertainty about the relation between side effects and phospholipidosis comes from studies using amiodarone. In addition to lipid accumulation, this drug also has several other side effects including bradycardia, congestive heart failure, pneumonitis, gastrointestinal intolerance, hepatitis, neurological effects, impaired vision, skin photosensitivity, blue skin, hypothyroidism, and hyperthyroidism (Vrobel et al., 1989). Such side effects may not be observed with other drugs such as chloroquine, which induces severe

retinopathy and myopathy (Hobbs et al., 1959; Whisnant et al., 1963). Another example is chlorphentermine, the use of which was discontinued because of its severe pulmonary and other unacceptable side effects (Ciborska et al., 1969).

In conclusion, the relationship between generalized drug-induced phospholipidosis and the other specific side effects produced by the drug has not been established. However, it has been well-documented that drug-induced ultrastructural myeloid bodies are present in all of the organs that are affected (Abraham et al., 1968; Fedorko et al., 1968a,b; Hruban et al., 1973; Wibo and Poole, 1974; Reasor et al., 1978; Kannan et al., 1989).

D. Pulmonary Macrophages

Our understanding of the pulmonary macrophage biology has been greatly facilitated by the relative ease with which they can be isolated. Macrophages can be isolated by alveolar lavage and cultured in the laboratory, and the responses can be studied relatively easily in laboratory animal models (Myrvik et al., 1961). A complete review by Reasor (1981) of all aspects of macrophage biology and CADs appeared a decade ago. Availability of human lung lavage samples also represents an excellent opportunity to study CAD-induced phospholipidosis (Martin et al., 1985; Martin and Standing, 1988).

Alveolar macrophages have several functions in addition to scavenging extraneous toxicants and endogenous waste such as surfactant material (Naimark, 1973; Geiger et al., 1975; Nichols, 1976; Fisher and Chander, 1985; Stern et al., 1986). They are involved in interactions with other cells of the immune system, principally lymphocytes, in the elicitation of cell-mediated immunity (Johnson et al., 1975; Moore and Myrvik, 1977; Hocking and Golde, 1979). Alveolar macrophages have an important role in the release of factors and chemical mediators that may modulate the migration of granulocytes and monocytes to the lung (Kazmierowski et al., 1977; Hunninghake et al., 1978; Hocking and Golde, 1979; Merrill et al., 1980), the induction of fibrogenesis, bronchoconstriction, vasoconstriction, and perhaps several other unknown functions. Several aspects of the alveolar macrophage biology including their origin and fate, ultrastructural features, physiology, and metabolism, their role in defensive mechanisms, and their adaptive responses have been well characterized (Cohen and Gold, 1975; Greene et al., 1977). Changes in macrophage function may predispose the host organism to increased disease susceptibility or tissue damage. A suspected cause of interstitial pneumonitis in amiodarone-treated patients is an alteration of the host immune system (Suarez et al., 1983; Akoun et al., 1984; Venet et al., 1985; Sandron et al., 1986), perhaps involving infiltration of alveolar macrophages and lymphocytes. The relationship among the molecular alteration in phospholipid metabolism, changes in membrane constitution by CADs, and signal transduction in the regulation of the immune system have not been established. However, it should be emphasized that any alteration in phospholipid metabolism, change in membrane phospholipid composition, and expression of several regulatory factors have a definite mechanistic link with the etiology of CAD-induced phospholipidosis (Turner and Kuo, 1985).

Pulmonary phospholipidosis is associated with the infiltration of foamy macrophages and lymphocytes (Vijeyaratnam and Corrin, 1972; Lullmann-Rauch and Scheid, 1975; Reasor, 1981; Heyneman and Reasor, 1986a; Ogle and Reasor, 1990). Extensive infiltration of macrophages was seen in chlorphentermine-treated rat lungs. Macrophages play a key role in the etiology of pulmonary phospholipidosis and have been extensively studied with regard to phospholipidosis (Vijeyaratnam and Corrin, 1972; Hruban et al., 1973; Karabelnik and Zbinden, 1975; Lullmann-Rauch and Scheid, 1975; Reasor, 1981; Heyneman and Reasor, 1986a,b).

Infiltration of alveolar macrophages in phospholipidosis, in general, is associated with increased phospholipid levels. Reasor et al. (1979) reported that chlorphentermine (30 mg/kg, ip) for 4 weeks resulted in an 18-fold increase in total phospholipids in macrophages. McNulty and Reasor (1981b) reported a 24.5-fold increase in total phospholipids in macrophages treated with iprindole (100 mg/kg, po) for 4 weeks. The cell size increases in proportion to the phospholipid accumulation for at least 4 weeks. The dose and response are proportional in most of the cases.

Alveolar macrophages treated with chlorphentermine, RMI 10,393, or chloramitryptyline contained large amounts of phosphoglycerides, sphingomyelin, and plasmalogens and small amounts of free fatty acids and cholesterol; no sulfides, triglycerides, or gangliosides were detected (Karabelnik and Zbinden, 1976). The compositional increase in various classes of phospholipids is important in understanding the structure-activity relationships between types of phospholipid being affected and various drugs. Reasor et al. (1979) also reported several other changes in alveolar macrophages in chlorphentermine-treated rats. In the case of amiodarone, phospholipidosis in macrophages has been associated with drug accumulation and distribution (Kirk et al., 1988, 1990). Alveolar macrophages of chlorphentermine-treated rats are susceptible to lipid peroxidation, and reduced glutathione plays a protective role (Reasor and Koshut, 1980). Phagocytic activity of cells, however, seems to be enhanced in vitro (McNulty and Reasor, 1981a,b).

Generally, it has been understood that the infiltration of macrophages is a characteristic of CAD-induced phospholipidosis. However, some exceptions do exist. Two independent studies were conducted in our laboratory to investigate the potency of chlorpromazine in inducing phospholipidosis in the lung and in macrophages of male Sprague-Dawley as well as Fischer 344 rats (Kodavanti

et al., 1991a,b). Consistently, we observed that chlorpromazine treatment decreased the number of alveolar macrophages despite its limited ability to increase macrophage phospholipid levels. Chlorpromazine may represent another unique class of drugs exhibiting rather specific effects on alveolar macrophages. Amiodarone may represent another class of drugs in this regard. It has been reported that, in patients receiving long-term amiodarone therapy, there is an infiltration of polymorphonuclear lymphocytes and other phagocytic cells but not macrophages (Martin et al., 1985; Myers et al., 1987). In addition to any specific effects on macrophages, effects on lymphocytes and other phagocytic cells in relation to phospholipidosis, as well as other cellular changes, need to be investigated. Work on alveolar macrophages might be of special significance, because these cells can be obtained from patients. Hence, any relationships between drug-induced phospholipidosis and effects on pulmonary macrophages might be of predictive and diagnostic value.

E. Phospholipids, Various Phospholipid Classes, and Tissue, Species, and Age Specificity

The distribution pattern of all phospholipids in the tissues depends on the structure and function of that particular tissue or organ. Likewise, the effects of CADs on a particular phospholipid are dependent on the tissue specificity for CAD accumulation. For example, the surfactant component of the lung is rich in disaturated phosphatidylcholine. It is known that drug-induced pulmonary phospholipidosis primarily results in elevation of this phospholipid (Ma et al., 1988; Reasor and Heyneman, 1983; Camus et al., 1989). Additionally, the severity of the effect on a particular class of phospholipid seems to depend on the type of CAD as well as on the tissue that is being affected (Lullmann et al., 1978; Matsuzawa and Hostetler, 1980d; Kacew, 1985; Pirovino et al., 1988). For example, a marked accumulation of neutral disaturated phosphatidylcholine, including cholesterol, cholesterol esters, and free fatty acids, occurs in macrophages isolated from 1-chloramitryptyline-treated rats (Karabelnik and Zbinden, 1975).

In the case of chlorphentermine and amiodarone-induced phospholipidosis, the specific type of phospholipid and the specific tissue in which such increases occur are of interest. Chlorphentermine-induced pulmonary phospholipidosis is associated with a maximum increase in disaturated phosphatidylcholine in the lung (Schmien et al., 1974; Gloster et al., 1976; Kacew, 1988). Also, fluoxetine, an inhibitor of serotonin uptake (Wong et al., 1975), induces accumulation of phosphatidylcholine in the lung, a property shared by chlorphentermine (Wold et al., 1976). A marked elevation of phosphatidylcholine occurs particularly in type II cells and alveolar macrophages and in the lung surfactant due to amiodarone and chlorphentermine treatment (Smith et al., 1973; Marchlinski et al., 1982; Reasor and Heyneman, 1983; Chatelain and Brotelle, 1985; Reasor et al., 1988). Amiodarone, chlorphentermine, and chlorcyclizine induce phospholipidosis in the lung, particularly in macrophages (Gloster et al., 1976; Gaton and Wolman, 1979; Kudenchuk, 1984; Chatelain and Brotelle, 1985; Reasor et al., 1988; Kodavanti and Mehendale, 1991). Other tissues also are affected but to a considerably lesser extent (Lullmann et al., 1978; Kannan et al., 1982; Mazue et al., 1984; Pirovino et al., 1988). Amiodarone-induced phospholipidosis in the liver is associated with a maximum increase in phosphatidylserine and phosphatidylethanolamine (Yap et al., 1987; Pirovino et al., 1988, 1990). Furthermore, it has been shown that amiodarone does induce cytoplasmic vacuoles, but not lamellar bodies, in the liver (Lambert et al., 1989; Pirovino et al., 1990). Certainly, additional evidence is necessary to confirm this finding.

Because amiodarone binds only to the hydrophobic moiety of the phospholipids (Joshi et al., 1988, 1989) and the ionic interactions of the polar moiety of the drug and a phospholipid are minimal, one can speculate that amiodarone is able to induce an accumulation of many kinds of phospholipids irrespective of ionic charges and/or the nature of the polar group of the phospholipids. On the other hand, chlorphentermine-phospholipid interactions occur mainly through polar, ionic moieties. Surprisingly, hydrophobic interactions are minimal and, predictably, chlorphentermine affects mainly phosphatidylcholine and the charged anionic polar lipids with which ionic interactions occur in the lung tissue (Joshi et al., 1989). Thus, understanding the ionic and hydrophobic interactions of CADs with phospholipids is important in explaining the tissue specificity of phospholipidosis.

Phospholipid fatty liver observed after chloroquine treatment has been extensively studied by Hostetler and his coinvestigators (Matsuzawa and Hostetler, 1980c; Hostetler et al., 1985). Chloroquine induces an accumulation of phosphatidylglycerol and phosphatidylinositol in various fractions of rat livers (Matsuzawa and Hostetler, 1980a,d). Chloroquine-induced phospholipidosis is less common and less prominent in the lung tissue. Chloroquine has a divalent cationic group in contrast to other CADs such as chlorphentermine and amiodarone. Lullmann and Wehling (1979) postulated that the tissue specificity and the specific class of phospholipid being affected by chloroquine is related to the presence of the divalent cationic moiety on this drug molecule. They proposed that the divalent cationic moiety possesses an unexpectedly high affinity for negatively charged polar lipids and, therefore, chloroquine has a remarkable tendency to induce an accumulation of gangliosides (Klinghardt, 1976), the anionic lipids such as bis(monoacylglycero)-phosphate, and phosphatidylinositol, all of which are present in relatively higher concentrations in the liver in comparison to the lung (Yamamoto et al., 1976; Tjiong et al., 1978). Similarly, gentamicin was reported to induce renal phospholipidosis, particularly

by increasing phosphatidylinositol in adult rats (Feldman et al., 1982; Wilmotte et al., 1983).

Gentamicin-induced lamellar bodies in humans have been associated with kidney toxicity (Kosek et al., 1974; Houghton et al., 1978; Smith et al., 1980). Gentamicin and amantadine induce phospholipidosis predominantly in the kidney (Feldman et al., 1982; Wilmotte et al., 1983; Kacew, 1985, 1987; Burmester et al., 1987). Gentamicin-induced phospholipidosis is associated with a maximum elevation of phosphatidylserine, phosphatidylcholine, and phosphatidylinositol detectable in kidney lysosomes as well as in rat whole kidney homogenates (Kacew, 1987). Chlorphentermine also exerted a maximum increase in phosphatidylinositol in the kidney, whereas the maximum increase in phosphatidylcholine occurred in lung tissue (Kacew, 1985). Because phosphatidylinositol can be affected the most (Feldman et al., 1982; Wilmotte et al., 1983), the deleterious long-term effects, particularly on regulation of signal transduction and protein phosphorylation, cannot be ignored. The relationship of phospholipidosis and other cellular changes will be discussed in "Mechanism of Phospholipidosis."

Brain is the other phospholipid-rich organ. The distribution of phospholipids in the white and gray matter of brain is variable. In general, brain is rich in phosphatidylethanolamine and lecithin (phosphatidylcholine), followed by sphingomyelin, phosphatidylserine, and phosphatidylinositol (Suzuki, 1976). It is well known that CADs interact with phospholipid membranes and regulate ion transport across membranes in the nervous system eliciting physiological responses (Levy and Richards, 1966; Patil, 1968; Seeman, 1977; Mason et al., 1984; Zychlinski and Montgomery, 1986; Nagai et al., 1987; Chatelain et al., 1989). Chloroquine- and amiodarone-induced neurotoxicity has been associated with phospholipidosis in the brain (Meier et al., 1979; Estes et al., 1987). There is very little evidence that a small degree of phospholipidosis could result in the brain following chronic CAD treatment (Klinghardt et al., 1981; Nilsson et al., 1981; Lemaire et al., 1982). However, lack of detailed information concerning phospholipidosis induced by CADs in the brain and its significance limits any interpretation of a relationship among tissue specificity, membrane action, and the mechanism of phospholipidosis in this organ. As far as other tissues are concerned, Wong and Hruban (1972) reported that testicular degeneration in chlorcyclizine-treated rats was associated with phospholipidosis-like alterations.

It should be noted that both the intensity of phospholipidosis-like ultrastructural alterations and the magnitude of chlorphentermine-induced phospholipidosis in the lung and adrenal gland were reported to be species specific (Lullmann-Rauch and Reil, 1974). Species specificity in the intensity of CAD-induced phospholipidosis may be due predominantly to the differences in capacity for metabolic elimination of CADs, either in the target or in the nontarget tissues. Metabolism of several CADs by the lung and liver has been shown to differ significantly between rats and rabbits (Ohmiya and Mehendale, 1980b, 1981, 1982; Young and Mehendale, 1986). Detailed studies have not been conducted on the phospholipidosis-inducing potency of several CADs in various species, types of phospholipids being affected, or the tissue specificity. A major factor that could play a role in species differences is the status of metabolic enzymes in target or nontarget tissues and the overall turnover of CADs by tissues in different animal species.

In an attempt to investigate the phospholipidosis-inducing properties of chlorphentermine and gentamicin in newborn rats, Kacew (1984) demonstrated that gentamicin induces phospholipidosis in kidneys, whereas chlorphentermine is effective in both the lung and kidney. Chlorphentermine or gentamicin, on the other hand, did not induce phospholipidosis in the lung or liver of chick embryos. Gentamicin exerted the greatest increase in phosphatidylinositol as compared to other classes of phospholipid in newborn rats, unlike in adults (Kacew, 1987; Kacew and Reasor, 1985). In comparing the CAD-induced phospholipidosis in newborn rats and in chick embryos, Kacew (1985) did not consider the possibility of the influence of an enzyme development pattern in newborns at different stages. Furthermore, the age-related effects in the drug-induced phospholipidosis may be due to differences in the enzyme profile and the structural components of the cell.

It is important to study the developmental aspects of phospholipid synthesis and catabolism pathways in the lung and other tissues with respect to different classes of phospholipids to understand the precise mechanism of phospholipidosis and several other effects of CADs.

F. Affinity of Drugs for Various Tissues, Intracellular Distribution, Pharmacokinetics, and Metabolism

The diversity in the pharmacological actions of the drugs known to induce phospholipidosis is also reflected in the diversity in uptake, distribution, metabolism, and excretion of these drugs. There is considerable evidence to indicate that pharmacokinetics and metabolism of drugs play an important role in the etiology of drug-induced phospholipidosis. For example, drugs that are rapidly metabolized fail to induce phospholipidosis (Joshi and Mehendale, 1989; Joshi et al., 1989). Furthermore, drugs known to induce pulmonary phospholipidosis may no longer induce phospholipidosis upon enhancement of their metabolism by prior administration of agents known to induce drug metabolism (Svendsen, 1977; Kacew et al., 1981; Kacew and Reasor, 1983). Likewise, inhibition of metabolism of some drugs may result in an increased potential for drug-induced phospholipidosis.

Various classes of CADs, such as adrenergics, antihistamines, antipsychotics, antimalarials, morphine-like analgesics, synthetic analgesics, anorectics, tricyclic

antidepressants, antiarrhythmics, adrenergic blocking agents, etc. (fig. 1), have been shown to accumulate selectively in the lung with lower concentrations found in the brain, liver, adipose, and other tissues (Brodie et al., 1950; Brown, 1974; Philpot et al., 1977; Bend et al., 1985). Preferential retention of CADs in the lung tissue has been attributed to a lower pH of the extravascular space in the lung tissue (Effros and Chinard, 1969; Nielson et al., 1981). In recent years, it has become increasingly evident that CADs have a special affinity for the neutral and acidic phospholipids which are predominant components of the surfactant in the lung tissue (Notter and Finkelstein, 1984; Fisher and Chander, 1985; Rooney, 1985; Dobbs, 1989; Tierney, 1989; Ruben et al., 1989). This affinity may be associated with the capacity of CADs to bind to phospholipids of the lung. The degree of phospholipid binding and the types of binding interactions have been studied recently using pure dipalmitoylphosphatidylcholine vesicles and concentric lamellar bodies (Joshi et al., 1988, 1989). A strong correlation is possible between the affinity of CADs for the lung tissue and their binding ability to a major constitutive phospholipid component of alveolar surfactant.

Biotransformation of drugs has important implications for the induction of phospholipidosis. Drugs may be metabolized to products that have a higher propensity for accumulation in tissues or, conversely, their accumulation may be vastly decreased by biotransformation. The uptake and metabolism of some classic CADs known to induce phospholipidosis have been investigated in our laboratory (Angevine and Mehendale, 1980a,b; Camus and Mehendale, 1986; Ohmiya and Mehendale, 1979, 1980a,b, 1981, 1982; Young and Mehendale, 1986). It has been reported that the lung possesses a surprisingly high capacity for the metabolism of some of the CADs (Brown, 1974; Ohmiya and Mehendale, 1980a,b, 1981, 1982). Isolated, perfused, and ventilated lung preparations have been extensively used to study the uptake, metabolism, and affinity of CADs for this organ. Chlorpromazine is rapidly taken up by the isolated perfused lung with a subsequent elimination of the nitrogen oxide metabolite in the perfusate, indicating that the lung is able to metabolize and rid itself of chlorpromazine (Ohmiya and Mehendale, 1982). These observations are consistent with the lack of phospholipidosis-inducing potency of chlorpromazine in vivo (Lullmann et al., 1978; Hruban, 1984).

Although in vivo acute administration of ^{35}S-chlorpromazine indicates that the drug is preferentially accumulated in the lung tissue of rats (Hackman et al., 1970; Bickel et al., 1983), in vivo phospholipidosis is minimal because of extensive nitrogen oxidation of this compound by the lung and because of the loss of affinity of the metabolite for the lung tissue (Ohmiya and Mehendale, 1980a, 1982; Beckett et al., 1988). Consistently, cellular systems that are unable to metabolize chlorpromazine do show phospholipidosis-like alterations when exposed to drugs (Drenckhahn et al., 1976; Lullmann et al., 1978; Hruban, 1984). In humans receiving chlorpromazine, two major metabolites have been detected, one is dehalogenated and the other is the nitrogen oxide form, in which promazine is a major metabolite in the plasma (Sgaragli et al., 1986). Thus, a lack of pulmonary retention due to nitrogen oxidation and dehalogenation appears to explain the lack of significant pulmonary toxicity seen after long-term chlorpromazine treatment. Reductive dechlorination of chloramphenicol by rat liver microsomes also occurs (Morris et al., 1983). Experiments in which the effects of inhibitors of metabolism on the ability of chlorpromazine to induce phospholipidosis are evaluated will be of considerable importance in understanding the role of drug metabolism in phospholipidosis. This approach has not been attempted because of the unavailability of a specific inhibitor of nitrogen oxidation in the lung tissue. Even when such an inhibitor becomes available, it should be devoid of toxic effects in the test animals as well as any undesirable interactions between the drug and the inhibitor.

Extensive nitrogen oxidation of imipramine by the rat lung is catalyzed by a flavin-containing monooxygenase, i.e., the same enzyme responsible for the nitrogen oxidation of chlorpromazine (Ohmiya and Mehendale, 1980b, 1981). The formation of a nitrogen oxide metabolite was not inhibited by classic cytochrome P-450 inhibitors such as SKF-525A and piperonyl butoxide, indicating that there is a flavin monooxygenase present in large quantity in the lung microsomes that is not associated with cytochrome P-450 (Ohmiya and Mehendale, 1981). Remarkable species variation has been observed in the pulmonary metabolism of imipramine and chlorpromazine (Drew et al., 1981). Rabbit lungs have minimal capacity to form the nitrogen oxide from imipramine and chlorpromazine, whereas rat lungs can metabolize these drugs to their nitrogen oxide (Ohmiya and Mehendale, 1980b, 1981). On the other hand, both rabbit and rat lungs possess the capacity to oxidize nitrogen, N-dimethylaniline, a reaction that is mediated by a flavin monooxygenase (Ohmiya and Mehendale, 1980b). These findings suggest the presence of more than one form of flavin monooxygenase in the rat lung and only one in the rabbit lung. Nitrogen oxides lack an affinity for lung or other tissues and are ultimately eliminated from the body. The explanation for the inability of imipramine and chlorpromazine to produce extensive phospholipidosis in vivo is their extensive metabolic elimination (Drenckhahn et al., 1976; Lullmann et al., 1978; Hruban, 1984; Joshi and Mehendale, 1989).

The importance of metabolism of drugs in drug-induced phospholipidosis also has been demonstrated by Kacew, Reasor, and other investigators (Svendsen, 1977; Kacew et al., 1981; Kacew and Reasor, 1983). They demonstrated that chlorphentermine-induced phospho-

lipidosis can be controlled or decreased by the simultaneous treatment with phenobarbital, a well-known inducer of drug-metabolizing enzymes. The significantly decreased phospholipidosis was associated with a marked increase in drug-metabolizing capacity (Kacew et al., 1981). Chlorphentermine has been demonstrated to undergo minimal metabolism (Dubnick et al., 1963, 1968; Lullmann et al., 1973). Presumably, the enhancement of the metabolism of chlorphentermine and, consequently, its accelerated elimination from the body are implicated as the mechanisms in this respect (Kacew et al., 1981).

Some CADs produce lipophilic metabolites that have the same affinity for the tissue as the parent drugs and also produce phospholipidosis (Holt et al., 1983; Adams et al., 1985; Camus and Mehendale, 1986; Young and Mehendale, 1986, 1987). Two such drugs have been extensively studied. Nor-chlorcyclizine is a nonpolar metabolite of chlorcyclizine that has a similar affinity for the lung tissue and induces phospholipidosis similar to that produced by chlorcyclizine (Kuntzman et al., 1965; Hruban et al., 1973; Blohm, 1979; Reasor, 1989). The other drug that is of current clinical concern regarding its pulmonary toxicity is amiodarone (Mason, 1987; Myers et al., 1987; Martin and Rosenow, 1988a,b; Vrobel et al., 1989). The principal metabolite, desethylamiodarone, formed mainly in the liver (Young and Mehendale, 1986), possesses greater affinity for the lung tissue (Camus and Mehendale, 1986) and is capable of greater toxicity (Kodavanti and Mehendale, 1991); it also is a stronger inducer of pulmonary phospholipidosis (Camus and Mehendale, 1986; Kodavanti and Mehendale, 1991).

Typically, it is believed that chlorinated drugs possess a high capacity for accumulation in the lung when compared to nonchlorinated congeners (Kuntzman et al., 1965; Brown, 1974; Morita and Mehendale, 1983b). Also, aromatic *p*-chlorination of some CADs blocks the much favored *p*-hydroxylation (Brown, 1974). *p*-Chlorination may also affect other pathways of metabolism. For example, Kruger et al. (1986) reported that hydroxylation and demethylation of imipramine by rat liver microsomes were greater than observed for chlorimipramine. Nitrogen oxidation, on the other hand, was more prominent with imipramine than chlorimipramine (Ohmiya and Mehendale, 1980b, 1981, 1984; Beckett et al., 1988). Decreased metabolic elimination of chlorimipramine owing to *p*-chlorination might have been expected to increase the metabolism of this compound via nitrogen oxidation. Also, it is important to note that nitrogen oxidation may represent the main process of elimination not only in the lung but also in the liver (Ohmiya and Mehendale, 1980b, 1981). As far as chlorinated and nonchlorinated analogs are concerned, little progress has been made in understanding the structure-activity relationship of various metabolic processes. More work in the area of metabolism of CADs and an understanding of how a specific metabolic alteration is associated with the phospholipidosis will help in understanding the relationship between drug metabolism and phospholipidosis.

Not all drugs known to be taken up in the lung induce phospholipidosis, although the lung does seem to be a major organ for uptake and accumulation of CADs. Propranolol possesses a high affinity for the lung tissue (Geddes et al., 1979), but its phospholipidosis-inducing property has not been well characterized. Clinical investigations have revealed that a major portion of this drug is accumulated in human lungs (Geddes et al., 1979). Propranolol uptake by the lung in vitro is competitive with other CADs (Dollery and Junod, 1976), and there is some evidence for an active uptake process (Kornhauser et al., 1980). Alveolar macrophages possess a specific high affinity for propranolol accumulation and the uptake has been noted to be active (Vestal et al., 1980). One may speculate that propranolol is a weak phospholipidosis-inducing agent, because this drug has only a weak affinity for phospholipids (Joshi et al., 1988, 1989). Alternatively, propranolol may also undergo metabolism to produce hydrophilic metabolites, thereby making it less effective in producing phospholipidosis (Nelson and Shetty, 1986).

II. Consequences of Phospholipidosis

A. Effects on the Metabolism of Biogenic and Exogenous Amines by Lung

CADs and biogenic amines share common transport mechanisms across the cell membrane, and thus, CADs may interfere with the uptake of biogenic amines (Morita and Mehendale, 1983a,b; Mehendale, 1984; Hart and Block, 1989). Clearance of exogenous and endogenous amines is a prominent non-respiratory function of the lung (Gillis, 1973; Bakhle and Vane, 1974; Smith et al., 1974; Gillis and Roth, 1977; Gillis et al., 1979; Angevine et al., 1982; Prasada Rao and Mehendale, 1987). The presence of 5-hydroxytryptamine in the endothelium of the lung may indicate its role in lung physiology. CADs interfere not only with the pulmonary uptake of 5-hydroxytryptamine but also with its metabolism to a nonreactive metabolite, 5-hydroxyindole acetic acid, by the monoamine oxidase system (Angevine and Mehendale, 1980a,b, 1982; Mehendale et al., 1983; Morita and Mehendale, 1983a,b; Mehendale, 1984; Zychlinski and Montgomery, 1985b).

Chlorphentermine, chlorimipramine, and their nonchlorinated analogs have been systematically studied for their effect on 5-hydroxytryptamine metabolism and uptake by lung in vivo and in vitro. The inhibition of 5-hydroxytryptamine uptake by these drugs has been studied using isolated rat and rabbit lungs (Angevine and Mehendale, 1982; Mehendale et al., 1983; Morita and Mehendale, 1983a,b).

The presence of chlorphentermine in the lung, in addition to inducing phospholipidosis, interferes with 5-

hydroxytryptamine uptake by the lung (Angevine and Mehendale, 1982; Mehendale et al., 1983). Chlorinated analogs which are taken up more effectively by the lung seem to affect 5-hydroxytryptamine transport and monoamine oxidases to a greater extent when compared to nonchlorinated drugs (Morita and Mehendale, 1983a). Interference of 5-hydroxytryptamine uptake and metabolism by chlorphentermine has been shown to be associated with the occurrence of pulmonary hypertension in patients receiving this anorexic drug (Lullmann et al., 1972; Harris and Heath, 1977). However, there is controversy regarding this assumption. Also, not all CADs are known to affect 5-hydroxytryptamine uptake and metabolism in the lung. For example, propranolol and chlorpromazine were shown to have no effect on the pulmonary clearance of 5-hydroxytryptamine and its metabolism (Morita and Mehendale, 1983a). Propranolol, however, has been reported to interfere with noradrenaline uptake by the heart (Foo et al., 1968). Chlorimipramine and chlorgyline affect the regional level of brain amines and their metabolism after chronic treatment in rats (Mousseau and Greenshaw, 1989). Effects of CADs on serotonin metabolism may influence serotonergic or adrenergic control of cellular responses. The effects of amiodarone and other CADs on serotonergic metabolism in the lung and other organ systems have not been well studied.

CADs interfere with the uptake of other exogenously added amine drugs that are structurally related (Angevine et al., 1982, 1984; Ohmiya et al., 1983; Kodavanti and Mehendale, 1991). Imipramine and chlorpromazine have been demonstrated to displace propranolol from the isolated perfused rabbit lung (Ohmiya and Mehendale, 1979; Ohmiya et al., 1983). Amiodarone displacement and kinetics in the lung were affected by chlorimipramine and promazine; however, propranolol did not have any effect (Camus et al., 1990). There are several such examples. Structurally related CADs presumably compete for the uptake of other drugs by the lung. The binding capacities to phospholipids vary considerably with the individual drug (Joshi et al., 1988). The drugs with greater binding and uptake are retained or are able to displace less tightly bound drugs and vice versa.

Drug-induced phospholipidosis, on the other hand, increases the uptake and affinity of the same or other CADs. For example, chlorphentermine-induced phospholipidosis results in increased uptake of other pneumophilic drugs (Angevine et al., 1982; Ohmiya et al., 1983). Recent observations of amiodarone uptake in amiodarone-induced phospholipidosis also indicate enhanced accumulation of amiodarone during phospholipidosis (Kodavanti and Mehendale, 1991). When amiodarone-induced phospholipidosis was not prominently manifested after 2 days of treatment, there was no increase in [^{14}C]amiodarone sequestration by the perfused lung from treated animals. However, [^{14}C]amiodarone uptake was increased in the isolated perfused lung when phospholipidosis became prominent (Kodavanti and Mehendale, 1991) upon continuation of amiodarone treatment.

These studies indicate that increased phospholipid levels increase the affinity of drugs for the lung tissue. Two possibilities can be suggested. First, the phospholipid-drug binding during phospholipidosis induction need not be saturated for phospholipidosis to be manifested. Second, the increase in phospholipids of the membranes may result in sufficient membrane alterations to open up additional binding sites for the drug. Chlorphentermine-induced pulmonary phospholipidosis not only increased the uptake of chlorphentermine but also increased the uptake of other CADs such as chlorpromazine and imipramine (Ohmiya et al., 1983; Angevine et al., 1984).

These observations have important clinical implications because drug-induced pulmonary phospholipidosis can be predicted to result in an enhanced pulmonary sequestration of other pneumophilic drugs. Such a sequence of events would potentially result in an acceleration of compromised non-respiratory and, possibly, respiratory functions. Clearly, many investigations need to be carried out to understand drug interactions with phospholipids in vivo.

B. Effects on Respiratory Functions of the Lung

Pulmonary affinity for CADs and induction of phospholipidosis have been well studied in the lung. However, the extent to which respiratory function is compromised has not been extensively examined. A systematic study was carried out to investigate whether phospholipidosis induced by chlorphentermine affected lung respiratory functions (Camus et al., 1989). Despite a massive induction of phospholipidosis by chlorphentermine, only minor effects were observed on lung mechanics. The only marginal effect evident was a slightly compromised recoil pressure in the lung. However, the effect on recoil pressure was abolished in histeresis experiments, indicating that alveolar filling with macrophages caused this effect (Camus et al., 1989).

Amiodarone-induced pulmonary dysfunction with restrictive type changes has been associated with a moderate decrease in the percentage of predicted forced vital and total lung capacities (Marchlinski et al., 1982; Veltri and Reid, 1985). Amiodarone treatment predisposes hamsters to pulmonary fibrosis (Cantor et al., 1984; Daniels et al., 1989). The pulmonary fibrogenic action of amiodarone after intratracheal instillation was studied by pathological examination, presence of hyperplastic type II cells, and synthesis and content of lung elastin (Cantor et al., 1987). Clinical experience with amiodarone also indicates significant evidence of pulmonary fibrosis (Marchlinski et al., 1982; Gefter et al., 1983). The relationship between CAD accumulation and amiodarone-induced pneumonitis, fibrosis, pulmonary hyper-

tension, and phospholipidosis need to be examined further in detail.

III. Mechanism of Phospholipidosis

A. Membrane Effects of Cationic Amphiphilic Drugs

Biological activities of drug molecules are most often dependent on their interactions with biomembranes. Even though the ultimate pharmacological effects may depend on drug-protein interactions, drug molecules must cross lipid membrane barriers before reaching the target site. Therefore, the interactions of drugs with phospholipids and phospholipid-containing membranes play critical roles in drug disposition and drug action. Because CADs contain lipophilic as well as hydrophilic moieties, their interactions with lipid membranes tend to be more complex.

1. Partition coefficients, transition temperatures, and defective structures in membranes. The mechanism of pharmacological actions of drugs resides in their membrane-binding properties. Because most CADs interact with phospholipids and phospholipid-containing membranes, it is important to consider the relationship between this interaction and phospholipidosis. Reactions of several neuroleptic CADs including phenothiazines and anesthetics on biological membranes have been known for a long time.

In an artificial bilayer of dioleoylphosphatidylcholine or in a biological membrane, the cationic group of CADs is normally placed between the polar head groups of phospholipids, and the hydrophobic portion is directed toward the hydrophobic interior of the membrane; thus, the drug molecule intercalates between lipid molecules (Seeman, 1972; Conrad and Singer, 1979, 1981; Kursch et al., 1983; Harder and Debuch, 1986).

The incorporation of drug molecules affects the physicochemical properties of the lipid bilayer such that the phase transition temperature from gel to liquid crystalline state may be altered (Seeman, 1972; Papahadjopoulos et al., 1975). CADs are known to decrease phase transition temperature (Seeman, 1972; Lee, 1978). The phase transition temperatures of phospholipids vary with molecular configuration, pK_a of the phospholipid, and pH of the surrounding medium. For example, the transition for dioleoylphosphatidylcholine occurs at 41°C; however, it is 44°C for phosphatidylglycerol (Kursch et al., 1983). For phosphatidylglycerol, the increase in transition temperature occurs at a lower pH, ranging from 41°C at pH 7 to 61°C at pH 2. The transition temperature, the pK_a values, and the membrane changes are measures indicative of the integrity of the membrane (Kursch et al., 1983). The intensity of drug effects on transition temperature is concentration dependent and varies with the individual drugs. A decrease in the transition temperature induces fluidization of membranes (Cullis et al., 1978). Decreased transition temperature, along with increased fluidization, alters ion channels, receptor configuration, and, ultimately, leads to a therapeutic or pharmacological response (Kanaho et al., 1981; Verkleij et al., 1982; Rooney and Lee, 1983; Welti et al., 1984; Luxnatt and Galla, 1986; Muller et al., 1986). Relatively higher drug concentrations are required to exert fluidizing effects on membranes. The presence of a halogen group on the hydrophobic domain of the drug molecule increases the fluidizing effect, the physiological and pharmacological potency, and phospholipidosis-inducing capacity of CADs (Seydel et al., 1981). The relationship between the membrane effects and phospholipidosis has not been studied systematically.

As may be anticipated from the diversity of their chemical structures, drugs vary in their lipid to water partition coefficients. High lipid solubility allows drugs to partition into the bilayer more effectively. Amiodarone has low water solubility, the lipid to water partition coefficient is 5.95 (the log value of the neutral form of the drug) and displays significant hydrophobic behavior (Warren et al., 1970). The drug alters lipid dynamics and the physiological state of normal membranes at micromolar concentrations (Chatelain et al., 1985). Fluorescence polarization (Chatelain et al., 1986) and fluorescence-binding studies of amiodarone with lipids have indicated that amiodarone partitions into the hydrophobic core of the lipid bilayer (Joshi et al., 1988, 1989). A strong correlation exists between binding of amiodarone, in vivo phospholipidosis-inducing potency of the drug, and its phospholipase-inhibiting capacity (Kodavanti and Mehendale, 1991).

The issue of the relationship between phospholipidosis induction and phospholipase inhibition or drug binding to phospholipids and their relative role in drug-induced phospholipidosis is mechanistically puzzling and will be considered in some detail. The antipsychotic drug, chlorpromazine, is extremely fat soluble and surface active (Seeman, 1977). At higher than therapeutic concentrations, chlorpromazine and other neuroleptics interact with membranes in a nonspecific way and fluidize all membranes, leading to enhanced spontaneous release of neurotransmitters (Seeman, 1977). The activity, at least in the case of neuroleptics, depends on the nature of the polar side chain as well as the hydrophobic ring structure (Seeman et al., 1974; Schwendener and Weder, 1978). It has been postulated that, if carbon 2 is attached to the methyl group on the polar side chain moieties, less activity is obtained. Also, if carbon 2 on the side chain is bound in a ring form, a decrease in activity is obtained (Gordon et al., 1963). A halogen substitution on the carbon-2 position on the hydrophobic ring, on the other hand, increases the physiological activity of phenothiazines (Gordon et al., 1963; Zirkle and Kaiser, 1980) and the hydrophilic interaction of drugs with phospholipids (Joshi et al., 1989). Thus, structure-activity relationships exist in CAD-membrane interactions. Further understanding of their actions on the phospholipids of mem-

branes and subsequent static or dynamic cellular interactions are important in examining the mechanism of drug-induced phospholipidosis.

In general, it has been concluded that halogenated drugs are more lipophilic (Leo et al., 1971; Cerbon, 1972); however, partition coefficients of chlorinated and nonchlorinated drugs are not comparable in terms of differences in their lipophilicity. Definitive conclusions with regard to the effects of halogenation and other substitutions affecting lipophilicity of CADs and the consequences on phospholipidosis are difficult because of limited information. Nevertheless, it can be stated that hydrophobic interactions of chlorinated and nonchlorinated drugs, which do not differ significantly between the two analogs (Joshi et al., 1989), may relate to their lipid to water partition coefficients rather than to their hydrophilic interactions.

2. *Hydrophobic and hydrophilic interactions of drugs with phospholipids.* The binding of cationic drugs to lipid bilayers (fig. 2) and the relationship between this binding and phospholipidosis has become an issue of controversy mainly because of the diversity of in vitro test systems and techniques used in such studies. As discussed in the previous section, it is apparent that CADs partition into artificial and biological membranes and induce defects in the membrane structure accompanied by alterations in membrane fluidity (Seydel and Wassermann, 1976; Lullmann and Wehling, 1979; Phadke et al., 1981; Verkleij et al., 1982; Kursch et al., 1983; Chatelain et al., 1986, 1989; Harder and Debuch, 1986; Kubo et al., 1986). Binding of drugs with membranes is reversible depending on the ionic charge of the drug and hydrophobicity of the bilayer, partition coefficient, pH, and pK_a of the amphiphilic molecules. Any change in the drug molecule structure alters the drug-phospholipid-binding profile. Drug-phospholipid-binding studies have been carried out using fluorescent probe techniques, originally described by Ma et al. (1985). The hydrophobic fluoroprobe, 1,6-diphenyl-1,3,5-hexatriene, is very useful in studying drug-phospholipid interactions because it positions between fatty acyl chains of phospholipids, changing the emission spectra (London and Feigenson, 1978; Ma et al., 1985). The competition between the probe and the drug molecule for the hydrophobic moiety on the phospholipid will depend on the strength of the hydrophobicity in a drug (fig. 3).

Fifteen structurally, pharmacologically, and mechanistically dissimilar CADs have been studied for their interaction with dipalmitoylphosphatidylcholine vesicles (Joshi et al., 1988; Joshi and Mehendale, 1989). Their phospholipid-binding potencies are also quite variable. Binding capacities and affinities of the drugs for isolated rat lung lamellar bodies showed similar concentration-response patterns in binding at the hydrophobic moiety, indicating that such binding to phospholipid membranes and organelles may occur in vivo. Some of the well-known phospholipidosis-inducing drugs did partition very effectively in the hydrophobic region of phospholipids, whereas others did not. For example, amiodarone partitioning with the hydrophobic region was very remarkable and was characterized by an equally high affinity. However, other phospholipidotic agents, such as chlorphentermine and chloroquine, did not partition into hydrophobic fatty acids of phospholipid bilayers. Such

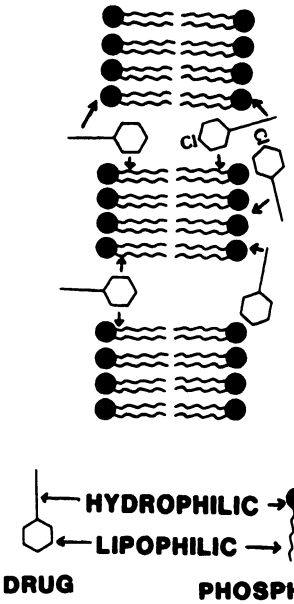

FIG. 2. Possible hydrophobic and hydrophilic interactions of CADs.

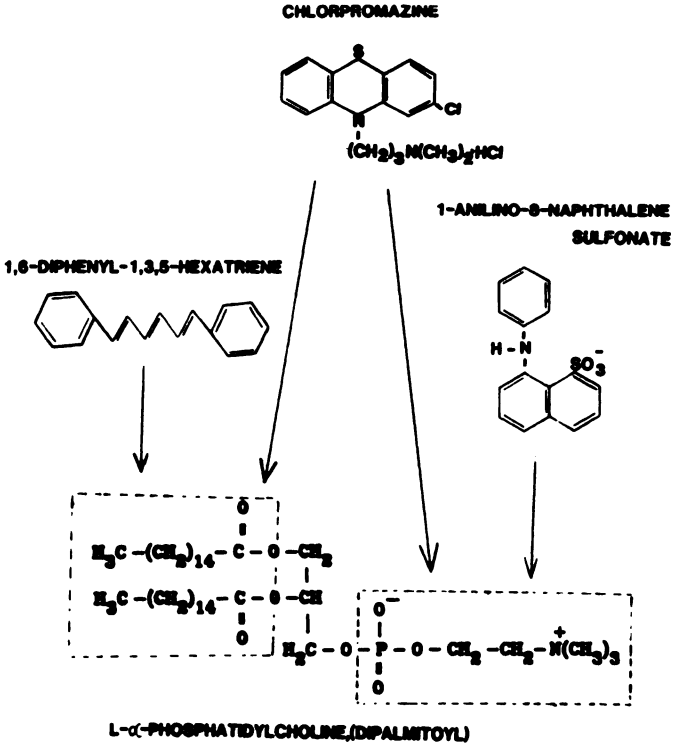

FIG. 3. Hydrophobic and hydrophilic interactions between fluorescent probes, an amphiphilic drug, and dipalmitoylphosphatidylcholine. Structure of chlorpromazine is given as an example of a CAD that can react with both sites on dipalmitoylphosphatidylcholine. Reproduced with permission from Joshi and Mehendale (1989).

findings suggest that any generalization based simply on lipophilicity and anticipated impact of structural modifications on lipophilicity will not permit an accurate prediction of the relationship between hydrophobic drug-phospholipid-binding phenomena and phospholipidosis.

Hydrophobic and hydrophilic interactions of drugs with phospholipids investigated using several other approaches (Frenzel et al., 1978; Ahmed et al., 1980) have also led to a conclusion that hydrophobic forces of drug and phospholipids will almost entirely determine the degree of binding for neutral lipids. This is in contrast with the negatively charged phosphatidylserine and gangliosides (Lullmann and Wehling, 1979), in which hydrophilic forces become important in binding to drugs. Although Lullmann and Wehling (1979) were not able to distinguish precisely between the role of electrostatic and hydrophobic forces in drug-phospholipid binding, they did speculate that both kinds of forces were involved in the binding of drugs to the phospholipid bilayer.

The hydrophilic interactions of several drugs with the neutral phospholipid, dipalmitoylphosphatidylcholine, also vary with the drug used. Our studies of hydrophilic interactions of drugs with dipalmitoylphosphatidylcholine were facilitated by the use of a fluorescent probe, 1-anilino-8-naphthalene sulfonic acid. This fluorescent probe has been extensively used to demonstrate several kinds of membrane interactions (Vanderkooi and Martonosi, 1971; Flanagan and Hesketh, 1973; Ma et al., 1985). 1-Anilino-8-naphthalene sulfonate does not give fluorescence signals with negatively charged phospholipids such as phosphatidylserine (Ma et al., 1985), but fluoresces intensely upon binding to the positively charged amino group in the hydrophilic region of the phospholipid (Ma et al., 1985). Cationic species such as Ca^{2+} and several monovalent cationic drugs have been shown to bind with the negative phosphate head group (Rojas and Tobias, 1965; Verkleij et al., 1982). Based on this, Ma et al. (1985) suggested that the binding of 1-anilino-8-naphthalene sulfonate with phospholipid vesicles should be augmented in the presence of CADs if the drug interacts with the net negative charge on the dipalmitoylphosphatidylcholine vesicles. They observed that fluorescence signals of the probe-phospholipid complex were intensified in the presence of calcium and chlorphentermine, a phospholipidotic drug (Ma et al., 1985).

The studies conducted with 15 drugs serve to illustrate that these drugs vary in their interaction with the hydrophilic moiety of phospholipids. A potent phospholipidotic drug, amiodarone, which shows intense hydrophobic interaction did not bind to the hydrophilic moiety (Joshi et al., 1988; 1989). Consistent with our observations, Chatelain et al. (1986, 1989) also concluded that amiodarone buries deeply in the hydrophobic core and is able to alter the physical characteristics of biological membranes.

In contrast to amiodarone, chlorphentermine does not bind to the hydrophobic moiety but instead exhibits binding with hydrophilic sites (Joshi and Mehendale, 1989; Joshi et al., 1988, 1989). Others have reported that chlorphentermine does participate in hydrophobic interactions to some extent (Ma et al., 1985). Gentamicin, an inducer of phospholipidosis in the kidney (Kacew, 1987), interacted mainly with the hydrophilic moiety of acidic phospholipid vesicles (Kubo et al., 1986). Interaction of acidic phospholipid substrates with gentamicin and other aminoglycoside antibiotics has been implicated as the cause of phospholipase inhibition and perhaps phospholipidosis (Mingeot-Leclercq et al., 1990a,b). Chlorphentermine, amiodarone, and gentamicin are examples of drugs that interact with either hydrophobic or hydrophilic moieties of phospholipids that might be implicated in drug-induced phospholipidosis.

Chloroquine does not interact with hydrophobic or hydrophilic moieties of dipalmitoylphosphatidylcholine (Lullmann and Wehling, 1979; Kubo and Hostetler, 1985; Joshi et al., 1988, 1989). It possesses a well-defined hydrophobic moiety akin to that of chlorphentermine. If hydrophobic interactions are of primary importance in the binding of drugs with phospholipids, one would expect that, regardless of the hydrophilic moiety on both of these drugs, they should partition into fatty acyl chains, because both of these molecules have fairly high lipid to water partition coefficients (Leo et al., 1971). However, ionic charges on polar side chains of drugs, as well as on the polar phospholipid molecules, seem to be of primary importance in drug-phospholipid interactions. In the case of chloroquine, the absence of hydrophilic interactions with dipalmitoylphosphatidylcholine vesicles was explained in terms of electrostatic repulsions of the two cationic amine groups on the polar side chain to the cationic amine of the neutral dipalmitoylphosphatidylcholine molecules (Lullmann and Wehling, 1979; Joshi and Mehendale, 1989).

Chloroquine interactions with the polar side chain of negatively charged phosphatidylserine and gangliosides suggest that an excessive overall positive charge might result in electrostatic repulsions with dipalmitoylphosphatidylcholine (Klinghardt, 1977; Drenckhahn and Lullmann-Rauch, 1978; Lullmann and Wehling, 1979). In an attempt to correlate the affinity of chloroquine with negatively charged phospholipids and its phospholipidosis inducing potency, some investigators have postulated that chloroquine differs to some extent from the monovalent CADs with respect to its effect on the ultrastructure of cytoplasmic inclusion bodies and distribution pattern of phospholipidosis (Gray et al., 1971; Klinghardt, 1977; Lullmann and Wehling, 1979). Furthermore, chloroquine has a particular tendency to induce the accumulation of gangliosides (Klinghardt, 1977) and anionic lipids, bis(monoacylglycero)phosphate and phosphatidylinositol (Tjiong et al., 1978; Frisch and Lullmann-Rauch, 1979). This suggests that the affinity of

chloroquine for anionic lipids, and its tendency to induce accumulation of such lipids in the tissues, may be due to the specificity of chloroquine binding. The lower efficacy of chloroquine to induce pulmonary phospholipidosis may be explained on the basis of relatively lower concentrations of anionic lipids in the lung (Lullmann and Wehling, 1979). The liver, which is relatively richer in anionic lipid composition, exhibits chloroquine-induced phospholipidosis (Lullmann and Wehling, 1979). It also is likely that, after the primary ionic interactions are established, the hydrophobic interactions depend on the nature of the aromatic structure.

The affinity of various CADs for amphiphilic phospholipids appears to involve many factors in addition to charge neutralization. It may depend on overall ionic interaction in hydrophilic and hydrophobic regions, the conditions of the medium, ionic charges of the medium, presence of cations or anions, and the nature of the buffers used. Although charge neutralization is important, the in vitro models used for the model studies may or may not be representative of the in vivo conditions.

Chlorpromazine exhibits a strong binding to hydrophobic as well as hydrophilic moieties of dipalmitoylphosphatidylcholine as evidenced by fluorescence probe studies, nuclear magnetic resonance, or equilibrium distribution of radiolabeled drug (Seydel and Wassermann, 1976; Lullmann et al., 1978; Lullmann and Wehling, 1979; Joshi and Mehendale, 1989; Joshi et al., 1989). Chlorpromazine increases 1-anilino-8-naphthalene sulfonate fluorescence more than 50-fold in a suspension of isolated lamellar bodies, indicating strong hydrophilic interactions (Joshi et al., 1989). In contrast, Di Francesco and Bickel (1977) reported that chlorpromazine binds only to the hydrophobic moiety on membrane phospholipids. The approach used by them might not have been sufficient to conclude that chlorpromazine does bind to polar phospholipids with hydrophilic interactions.

Similarly, imipramine also exhibits strong hydrophilic and relatively weak hydrophobic interactions (Joshi and Mehendale, 1989; Joshi et al., 1989). Both imipramine and chlorpromazine induce phospholipidosis-like alterations in vitro but fail to do so in the lung in vivo (Lullmann et al., 1978; Hruban, 1984). The explanation for this difference may lie in the metabolism and elimination of these drugs. Both of these drugs are extensively metabolized to polar metabolites, with loss of affinity for the membrane (Ohmiya and Mehendale, 1979, 1980a,b, 1981, 1982, 1984). It could be speculated from our binding studies that, if a drug displays binding to both hydrophobic and hydrophilic moieties of the phospholipid, the metabolic elimination is relatively higher. Thus, chlorpromazine, imipramine, and propranolol are examples of drugs that bind to both hydrophobic and hydrophilic sites of phospholipids. These drugs are also known to be extensively metabolized. Amiodarone, cyclizine, chlorcyclizine, and chlorphentermine bind only to either the hydrophobic or the hydrophilic moiety (Joshi and Mehendale, 1989; Joshi et al., 1989) and exhibit minimal metabolism (Kuntzman et al., 1965; Dubnick et al., 1968; Brown, 1974; Young and Mehendale, 1986). This concept needs further verification.

One additional issue needing attention in this section is the binding of halo-substituted or nonhalogenated drugs to phospholipids. It has long been believed that halogen substitution at a critical carbon atom on a nonpolar ring structure makes an amphiphilic drug more hydrophobic (Leo et al., 1971). This may be true in some instances. However, this does not seem to be true if we compare octanol to water partition coefficients of the halogenated and nonhalogenated drugs (imipramine, chlorimipramine; promazine, chlorpromazine; phentermine and chlorphentermine). In fact, some of the drugs (e.g., chlorimipramine versus imipramine) containing a halogenated group actually have smaller octanol to water partition coefficients than do the nonhalogenated parent compounds (Leo et al., 1971).

If a high lipid to water partition coefficient is an index of hydrophobicity, chlorinated analogs should have considerably higher lipid to water partition coefficients than their parent compounds. Although the chlorinated drugs interact with phospholipids more intensely, in contrast to the anticipated augmentation of hydrophobic binding, we have observed highly intensified hydrophilic interactions (Joshi and Mehendale, 1989; Joshi et al., 1989). Therefore, the difference between chlorinated and nonchlorinated drugs in interacting with phospholipids is due to an augmentation of hydrophilic binding. Chlorinated and nonchlorinated drugs react with hydrophobic moieties with similar intensity, as illustrated by chlorpromazine and promazine (Joshi et al., 1989). Interestingly, the report from Kanaho et al. (1981) indicated that increased affinity of the drugs (cationic phenothiazines) for the plasma membrane by halogen substitution may involve the polar head group of the phospholipid membrane. The halogen atom, which is incorporated at position 2 on a hydrophobic ring, has an electron-withdrawing property. This property of the halogen may tend to withdraw electrons from the adjacent carbon ring and make the ring more electropositive (Bloom and Laubach, 1962; Gordon et al., 1963; Zirkle and Kaiser, 1980). Such a molecular change on a drug can alter the electronic configuration of a drug molecule. Electropositivity of a ring moiety of a drug might be involved in increased attraction to the negatively charged oxygen head group on dipalmitoylphosphatidylcholine. Interactions of halogenated and nonhalogenated drugs with negatively charged phospholipids, therefore, might be of greater interest in understanding the role of the polar head group and changes in the nonpolar moiety of a drug molecule. Possible interactions of CADs with the phospholipid bilayer are depicted in fig. 2.

3. Structure-activity relationship for cationic amphi-

philic drug-induced vacuoles and lamellar body formation. The appearance of clear cytoplasmic vacuoles (drug storage site) has been reported for some CADs (Ruben et al., 1985, 1989; Rorig et al., 1987; Ruben, 1987). Ruben and his coinvestigators established a structure-activity relationship for the development of clear cytoplasmic vacuoles, based on their studies on disobutamide and its structural analogs. It is obvious from their work that clear cytoplasmic vacuoles and lamellar bodies are two different manifestations of CAD action. Although the cationic amine group and its basicity are essential for the development of cytoplasmic vacuoles, the lipophilicity of molecules does not appear to play a significant role. Furthermore, two basic amines on the hydrophilic side chain are necessary. This seems true for chloroquine, because this drug also has two basic amines and is capable of producing clear cytoplasmic vacuoles. Unlike clear cytoplasmic vacuoles, lamellar bodies appear with CADs having one or two basic amines on the hydrophilic side chain. Moreover, the lipophilic nature of CADs is very essential for the development of lamellar bodies. The drugs with two basic amines and a well-defined lipophilic region, such as disobutamide and chloroquine, are capable of inducing a mixed type of structures with lamellar bodies and clear cytoplasmic vacuoles. The drugs with a well-defined lipophilic region and only one basic amine on the side chain, such as imipramine and amiodarone, are capable of developing only lamellar bodies. Unlike disobutamide, the halogen substitution on CADs with one basic amine makes the drug more potent in inducing lamellar bodies. Also, we have noted that halogen substitution on a lipophilic ring structure increases the affinity of the drug for polar phospholipids and lamellar bodies (Joshi and Mehendale, 1989; Joshi et al., 1988, 1989).

It is believed that clear cytoplasmic vacuoles are drug storage sites and the tissues with vacuoles do show elevation of phospholipids. The presence of phospholipids also has been noted in these vacuoles. However, to what extent the vacuoles contribute in overall phospholipidosis is far from clear. Usually, vacuolation is accompanied by the appearance of lamellar bodies. Although the cytoplasmic vacuoles and lamellar bodies are characteristics of CAD-induced phospholipid storage, these are strictly different in nature and obey strong structure-activity relationships. Thus, these studies do shed some light on the mechanism of the development of cellular structures. However, several questions remain to be addressed: (*a*) why these structures do not seem to alter cell function, (*b*) at what degree of these changes is the cell function affected, (*c*) what is their pathobiological significance, and (*d*) what is the threshold between normal physiological range and toxicity?

B. Lysosomes, Phospholipid Metabolism, and Cationic Amphiphilic Drugs

Lysosomes are important in the etiology of phospholipidosis because it is now established that some CADs concentrate in the lysosomes and inhibit the intralysosomal breakdown of phospholipids (Reijngoud and Tager, 1976; Ohkuma and Poole, 1978; Tulkens and Trouet, 1978; Hostetler et al., 1985). Whether CAD-induced clear cytoplasmic vacuoles and the hydrolytic activity inside vacuoles interfere with phospholipid metabolism is not known. It has been understood that the acid milieu of the lysosomes is conducive to the predominant ionization of pneumophilic drug molecules. Major effects of CADs on both phospholipid metabolism and the accumulation of lamellated bodies have been attributed to an altered lysosomal metabolism.

There has been considerable interest and debate concerning the subject of how CADs interfere with phospholipid catabolism in the lysosomes. De Duve et al. (1974) and others (Wibo and Poole, 1974) have shown that weak bases, including certain drugs and dyes, accumulate in the lysosomes. Weak organic bases of an amphiphilic nature cannot pass through the lipid phase of the membrane when the compound is in its ionized form. Ionization of a drug depends on the hydrogen ion concentration and the pK_a of the base. For most basic dyes, the pK_a falls in the range of 8 or greater. Because lysosomes have a markedly lower interior pH, CADs that are basic, i.e., have a pK_a in excess of 7 or 8, maximally concentrate in the lysosomes (Lullmann et al., 1978). Thus, CADs find their most favorable environment within lysosomes containing anionic lipids.

Lysosomal accumulation of chloroquine in very high concentrations (mM) has been noted in vivo and in vitro (Allison and Young, 1964; Ohkuma and Poole, 1978; Hollemans et al., 1981; Poole and Ohkuma, 1981; Hostetler et al., 1985). Accumulation of chloroquine in the lysosomes also has been studied using pH-sensitive fluorescent dyes (Ohkuma and Poole, 1978). In the case of chlorpromazine, rapid accumulation was shown to occur inside the negatively charged membrane vesicles and a concentration of more than two orders of magnitude greater than its exterior concentration was observed (Bally et al., 1985). In contrast, amiodarone does not seem to be lysosomotropic (Heath et al., 1985), raising the question of why some CADs are lysosomotropic and others are not? A partial explanation may come from the studies of disobutamide-induced clear cytoplasmic vesicles. Amiodarone, unlike disobutamide and chloroquine, has only one basic amine, and the hydrophilic portion does not react with polar phospholipids. On the other hand, lipophilic interactions of amiodarone and phospholipids as well as lamellar bodies are prominent. Therefore, amiodarone does not meet the structural requirement to be stored in the acid compartment of the cell.

Drugs exert several effects on the lysosomes and lysosomal enzymes. Lysosomal stabilizing effects of chlorphentermine have some functional implications in edema (Merkow et al., 1971; Lullmann et al., 1975; Reasor and

Walker, 1979; Townsley et al., 1985; Wohns et al., 1985; Fazekas and Szekeres, 1988; White et al., 1988). Lullmann et al. (1975) found that the formation of paw edema induced by dextran in rats was inhibited by chronic pretreatment with amphiphilic drugs. These effects were attributed to the stabilizing effects of drugs on the lysosomes resulting in reduced drug availability to produce a response (Reasor and Walker, 1979). Chloroquine, a phospholipidotic drug (Abraham et al., 1968; Lullmann et al., 1975), also has been shown to stabilize lysosomal membranes (Weissmann, 1966; Wibo and Poole, 1974) in vivo and in vitro.

CAD-induced accumulation of lamellar bodies in various cell types also has been attributed to metabolic changes in the lysosomes. Lamellar bodies possess several similarities to lysosomes. Lullmann-Rauch and Watermann (1987) reported that lipid storage lysosomes (lamellar bodies) in renal duct cells and hepatocytes of rats, induced by CADs, retain the ability to fuse with autophagosomes/autolysosomes.

C. Inhibition of Phospholipases

Depletion of major membrane phospholipids in situ can occur by the action of phospholipases (A_1 and A_2), lysophospholipases, and diesterases and/or by direct hydrolysis mediated through phospholipase C (Stryer, 1988). Lysosomal phospholipase C type activity against sphingomyelin, phosphoinositol, and other phospholipids is well documented (Matsuzawa and Hostetler, 1979, 1980a). CADs accumulate in the lysosomes and inhibit phospholipases (Brindley et al., 1975; Defrise-Quertain et al., 1978; Gaton and Wolman, 1979; Heath and Jacobson, 1980b; Matsuzawa and Hostetler, 1980b; Hostetler and Matsuzawa, 1981; Beckman et al., 1982; Kunze et al., 1982; Zborowski and Brindley, 1983; Hostetler, 1984; Kubo and Hostetler, 1985; Heath et al., 1985; Fredman et al., 1986; Grabner, 1987; Baumann et al., 1987; Shaikh et al., 1987; Hostetler et al., 1988; Kacew, 1988; Martin et al., 1989).

Two different proposals were made regarding the mechanism of phospholipidosis. One of these basically comes from Lullmann and his coinvestigators' work on the binding of CADs to phospholipids (Lullmann et al., 1978). In our study, we also have determined that CADs bind to phospholipids and isolated lamellar bodies of the lung with hydrophobic and hydrophilic moieties (Joshi et al., 1988, 1989). Lullmann and his coinvestigators hypothesized that, after the binding of drugs to phospholipids, drug-phospholipid complexes are formed that are resistant to phospholipase action, resulting in a buildup of phospholipids in the lysosomes (Lullmann et al., 1978). Several binding studies, including our approach which involved 15 different CADs and phospholipids as well as lamellar bodies, are in good correlation with the ability of various drugs to induce phospholipidosis. Drug-phospholipid complexes are unstable. Hence, the binding and the effects are reversible after discontinuation of treatment or removal of the drug from the incubation medium. This is not an insignificant consideration, because this aspect of the interaction makes it difficult to obtain direct evidence for the proposal that drug-phospholipid complexes are unsuitable substrates for phospholipases.

Although drug binding to phospholipids and its phospholipidosis-inducing potency are in good correlation, the inhibition of phospholipases in vitro also correlates well with a drug's ability to induce phospholipidosis (Hostetler, 1984; Kubo and Hostetler, 1985; Martin et al., 1989). Inhibition of phospholipases as the cause of drug-induced phospholipid accumulation has been suggested as an alternative hypothesis for the mechanism involved in phospholipidosis. Consistent with this hypothesis, amiodarone, a strong phospholipidotic drug in humans and in animal models, is a potent inhibitor of lysosomal phospholipases in vivo and in vitro (Heath et al., 1985; Hostetler et al., 1986, 1988; Shaikh et al., 1987; Martin et al., 1989; Kodavanti and Mehendale, 1991). Inhibition of hepatic triglyceride lipase activity in rats was attributed to hypothyroidism associated with amiodarone therapy (Kasim et al., 1987) because supplementation with thyroxine abrogated this effect.

Purified rat liver lysosomal phospholipase A_1 has been reported to be inhibited by chlorpromazine, propranolol, and chloroquine in vitro (Hostetler, 1984; Kubo and Hostetler, 1985; Grabner, 1987). To evaluate the mechanism of phospholipase inhibition, Kubo and Hostetler (1985) conducted substrate and drug saturation kinetic experiments in vitro using chloroquine and chlorpromazine. The purpose was to evaluate whether the drug is bound to the enzyme, thereby making an enzyme nonreactive to the substrate, or whether the drug is bound to the substrate, thereby making the substrate nonreactive to the enzyme. Either may be the case and the result would be a decreased breakdown of phospholipids. Most of the inhibition studies are carried out in in vitro situations, which involve addition of bolus amounts of drug into the incubation medium containing enzyme and the substrate. If the purpose of the study is to determine whether phospholipase inhibition was due to binding of the drug to the enzyme or due to the binding of the drug to the substrate phospholipid, in vitro incubations may not be the appropriate way unless special kinds of substrate saturation kinetics are employed in some instances. Perhaps, saturation kinetics may be the only valid in vitro approach, as demonstrated for chloroquine which, despite not binding to dioleoylphosphatidylcholine, inhibits phospholipase A and C (Matsuzawa and Hostetler, 1980b; Hostetler and Matsuzawa, 1981; Kubo and Hostetler, 1985). However, although chloroquine does not bind to dioleoyl- and dipalmitoylphosphatidylcholine (neutral phospholipids) (Kubo and Hostetler, 1985; Joshi et al., 1988, 1989), it is known to bind to negatively charged phospholipids such as phosphatidylserine and gangliosides (Klinghardt, 1977; Drenckhahn

and Lullmann-Rauch, 1978; Lullmann and Wehling, 1979). Therefore, in vivo, chloroquine may yield an outcome not predicted by the in vitro studies.

Association of chlorphentermine with pulmonary phospholipids, but not phospholipases, in vivo has been believed to be the mechanism involved in phospholipidosis (Ma et al., 1988). In this investigation, the association of chlorphentermine with phospholipids was studied by quantitating radioactive drug in the lipid fraction of the lung tissue. There may be a technical problem in interpreting these results because the drug is not covalently bound to the phospholipids or the drug may be coeluted with lipids causing an artifact. This was of particular concern in our studies of amiodarone uptake experiments (Kodavanti and Mehendale, 1991). We observed that the 2:1 mixture of chloroform:methanol used for the extraction of lipids also extracted free (unbound) amiodarone from the aqueous solution (Kodavanti and Mehendale, 1991). If such a likely situation prevails for chlorphentermine, no conclusion can be drawn from the above study of the association of chlorphentermine with the lipid fraction. In our studies, not only chloroform:methanol extracted 100% of the unbound amiodarone from the aqueous phase, the free drug also migrated along with dipalmitoylphosphatidylcholine in the thin layer chromatographic separation of various phospholipids (Kodavanti and Mehendale, 1991). Because of this kind of inherent technical problem and the characteristic reversibility in drug binding to phospholipids, it is difficult to rule out the possibility that the association of drugs with phospholipids in vivo could be involved as a mechanism rendering the phospholipid-drug complex an unsuitable substrate for phospholipases. Although one should consider that drug binding to phospholipids in vitro is strongly correlated with their in vivo phospholipidosis-inducing potency, the obtaining of more convincing direct evidence would be desirable.

Hostetler and his coinvestigators studied the inhibitory effects of several CADs on lysosomal phospholipases A and C isolated from the rat liver and lung (Hostetler and Matsuzawa, 1981; Hostetler et al., 1986). Amiodarone was shown to be the most potent inhibitor of phospholipases in vitro (Hostetler et al., 1986; Martin et al., 1989). Except for the drug amiodarone, there is a correlation between drug concentration and inhibition of phospholipases in the lysosome. Although amiodarone does not seem to be highly lysosomotropic, Hostetler et al. (1986) suggested that the effect still can be seen because of the high potency of this drug to inhibit phospholipases at very low concentrations. Additional studies are needed to evaluate the intracellular distribution of amiodarone. Autoradiographic analysis of intracellular distribution and other approaches such as fluorescent probe measurements of drug concentrations might provide likely answers.

Grabner (1987) postulated that imipramine and ambroxol influence phospholipase A_2 activity by decreasing the transition temperature of the phospholipid substrate, because the effect of imipramine on the temperature profile of the phospholipase A_2 activity is very similar to its action on the phase transition profile (Kursch et al., 1983). A correspondence of the temperature activity profile of phospholipase A_2 in relation to phospholipid hydrolysis was shown for phospholipase A_2 from porcine pancreas (Op den Kamp et al., 1975; Goormaghtigh et al., 1981), bee venom (Upreti and Jain, 1980), and platelets (Kannagi and Koizumi, 1979). Grabner (1987) observed that the hydrolytic potency of phospholipase A_2 is not substantially influenced by imipramine and ambroxol. These studies suggest that CADs interfere with the membrane bilayer structure and may not alter the phospholipase properties by binding to the enzyme. Ma et al. (1985) also favor this idea that the inhibition of phospholipase A_2 in the presence of chlorphentermine is due to the formation of a drug-phospholipid complex, making the complex an unsuitable substrate for phospholipases.

Hostetler and his coinvestigators (Matsuzawa and Hostetler, 1980b; Hostetler and Matsuzawa, 1981; Hostetler, 1984; Kubo and Hostetler, 1985, 1987; Hostetler et al., 1986, 1988) suggested that drugs complex with enzymes and inhibit phospholipases. Kinetically, it will be difficult to prove that the enzyme was or was not affected simply by measuring the activity. We have observed this phenomenon in our drug-binding studies in which amiodarone binding to dipalmitoylphosphatidylcholine at a hydrophobic site was saturated when there was still half the quantity of amiodarone remaining as free drug (Joshi et al., 1988).

There are only a few reports concerning the in vivo inhibition of phospholipases. Inhibition of phospholipase A by gentamicin and phospholipase C inhibition by chlorphentermine were studied (Kacew, 1987, 1988). However, the effects of a large number of other drugs are not known and these investigators used crude homogenates for the estimation of phospholipases. Chlorphentermine in vivo was able to inhibit phospholipase A and C of alveolar macrophages without any effect on lung lysosomal phospholipase (Kodavanti et al., 1991b). Recent studies have revealed substantial information regarding amiodarone- and desethylamiodarone-induced in vivo inhibition of phospholipases in the lung. Phospholipases of the lung lysosomes were inhibited with transient recovery during the treatment (Kodavanti and Mehendale, 1991). The inhibition of macrophage phospholipases was substantial with no recovery in activity during the treatment.

Although our studies with amiodarone indicate a strong correlation between phospholipase inhibition and phospholipidosis, it is difficult to explain the actions of all CADs based only on this mechanism. Also, the specificity of phospholipases for substrate, type of phospho-

lipid substrate being affected, proportion of substrates present in tissue, and the role in metabolism of phospholipids should be considered with care (Heath and Jacobson, 1980a,b). The de novo pathways of phosphatidylcholine synthesis in the lung produce principally unsaturated molecules (Possmayer et al., 1977; Rooney and Wai-Lee, 1977) that must be converted to dipalmitoyl species (saturated) by a remodeling process (Brumley and van den Bosch, 1977) involving phospholipase A_2 action. Under normal conditions, the dipalmitoylphosphatidylcholine is not degraded by these enzymes (Heath and Jacobson, 1980a). Therefore, it is difficult to envision a mechanistic role for CAD inhibition of phospholipase A_2 in CAD-induced phospholipidosis, especially because an increase in unsaturated phospholipids has not been reported. The substrate specificity and types of phospholipids being affected by CADs should be considered thoroughly before invoking inhibition of any particular phospholipase as a generalized mechanism of drug-induced phospholipidosis.

D. Effects of Cationic Amphiphilic Drugs on Phospholipid Synthesis

One can postulate that the mechanism of CAD-induced phospholipidosis should either involve decreased degradation or increased synthesis of phospholipid(s). Some critical examples from in vitro and in vivo studies are available to suggest that CADs do have effects on phospholipid synthesis. In human skin fibroblasts, chloroquine in vitro stimulates incorporation of precursor components into fatty acid, phospholipid, and cholesterol and stimulates their synthesis (Chen et al., 1986). In the rat liver, chloroquine in vitro has been shown to increase glycerol incorporation into phospholipids within the microsomes as well as glycerol transport to the lysosomes (Matsuzawa and Hostetler, 1980a). However, the mechanism by which chloroquine stimulates phospholipid synthesis and transport is not clear. Although tentative, these observations permit us to infer that chloroquine-induced phospholipidosis may be due to increased phospholipid synthesis (Reasor and Hostetler, 1984). Chlorpromazine, which has minimal phospholipidosis-inducing potency, affects the intermediary metabolism of phospholipids by inhibiting 1-acylglycerol-3-phosphate acyltransferase (Yada et al., 1986). Chlorpromazine also affects phospholipid synthesis in vitro. Leli and Hauser (1987) reported that chlorpromazine, desmethylimipramine, propranolol, and other CADs may affect phospholipid metabolism by inhibiting cystosine triphosphate-phosphocholine cytidyltransferase, thus decreasing incorporation of precursors into phosphatidylcholine and phosphatidylethanolamine or inhibiting phosphatidic acid phosphohydrolase and thus stimulating synthesis of acidic phospholipids by inositol exchange reactions. Karabelnik and Zbinden (1976) observed an inhibition of phospholipid synthesis in the rat lung in vivo, as measured by incorporation of [^{14}C]palmitic acid into phospholipids, by chlorphentermine and RMI 10,393. However, significant stimulation of [^{14}C]palmitate incorporation into phospholipids was seen with another phospholipidotic agent (RO 4–4318). The authors suggested that chlorphentermine and RMI 10,393 induce phospholipidosis by decreasing phospholipid degradation, whereas RO 4–4318 induces phospholipidosis by increasing synthesis. In accord with these observations, it was also reported that chlorphentermine does not increase phospholipid synthesis in the rat lung (Gonmori et al., 1986).

Results of the above reports suggest that CADs have specific effects on various enzyme systems involved in phospholipid synthesis and catabolism. No generalization among all CADs can be made relative to their effects on the enzymes involved in synthesis of phospholipids. The effects may vary with the type of cell system or tissue, species, duration of drug exposure, and the dose level used in the study. Additional evidence is needed to establish whether there is a structure-activity relationship among various CADs in relation to their effects on phospholipid synthesis.

E. Correlations: Partition Coefficients, Affinity for Tissues, Cationic Amphiphilic Drugs-Phospholipid Interactions, Pharmacokinetics, Inhibition of Phospholipases, and Phospholipidosis

Drug-induced phospholipidosis is a manifestation of the interaction of CADs at the tissue, cellular, and molecular level. In table 1 a graded evaluation of various mechanistic aspects of drug-induced phospholipidosis is presented. Based on the selected criteria listed in the table, an outline describing phospholipidosis-inducing capacity of drugs is given in fig. 4. Because most of the details have been presented in previous sections, only a few major points important in understanding phospholipidosis are reconsidered here.

It is clear from table 1 that binding of CADs with phospholipids and inhibition of phospholipases are correlated with their ability to induce phospholipidosis. The octanol to water partition coefficient does not correlate with phospholipidosis, nor does drug binding correlate with hydrophobic and hydrophilic moieties of phospholipids. Metabolically inert drugs, which bind with phospholipid on either the hydrophobic or hydrophilic moiety, seem to be effective (fig. 4). Furthermore, specificity of the phospholipid affected by drugs depends on the nature of the cationic side chain and hydrophobic moiety of drugs. The information on comparative effects of chlorinated and nonchlorinated drugs is not available for phospholipase inhibition. Such a comparative study would be beneficial in further defining the role and mechanism of CAD-induced phospholipidosis. Based on the available information regarding the two hypotheses, one cannot suggest either mechanism as a generalization for all CADs. The formation of a drug-phospholipid complex as an unsuitable substrate needs careful scrutiny

TABLE 1

*Comparison of toxicological and metabolic properties of several amphiphilic drugs**

Name of drug	Phospholipidosis in vivo†	Binding to lamellar bodies in vitro		Affinity for lung‡	Metabolic elimination§	Inhibition of phospholipases (IC_{50}, mM)‖		Lipid/water partition coefficient¶
		Hydrophobic	Hydrophilic			A	C	
Amiodarone	3	3	0	3	<1	0.016		
Desethylamiodarone	3	3	0	3				
Phentermine	0	0	<1	1	1			2.1
Chlorphentermine	3	0	>1	2	<1	0.21	0.45	2.26
Chloroquine	3	0	<1			0.3	0.33	4.63
Promazine	0	3	2	<2	3			4.55
Chlorpromazine	0	3	3	2	3	0.03	0.07	5.32
Imipramine	1	1	2	>2	3	0.23	0.25	4.62
Chlorimipramine	2	0	3	3				3.88
Trimipramine		2	2					
Cyclizine		0	1	2	1			
Chlorcyclizine		0	2	3	<1			3.98
Promethazine		3	2		2			
Chloramphenicol		3	0	2				1.14
Propranolol	<1	2	<2	2	2	0.25	0.38	3.14

* The tabulation for phospholipidotic potency, binding capacities, affinities for the lung tissue, and metabolic elimination are given in the order of increasing effectiveness. 0, no effect; 1, mild effect; 2, moderate effect; 3, maximum effect. Adapted in part from Joshi et al. (1989.)

† References: Lullmann et al., 1978; Hostetler and Matsuzawa, 1981; Ohmiya et al., 1983; Hruban, 1984; Kodavanti and Mehendale, 1991.

‡ References: Dubnick et al., 1968; Brown, 1974; Ohmiya and Mehendale, 1980b, 1982, 1984; Camus and Mehendale, 1986; Heyneman and Reasor, 1986b.

§ References: Dubnick et al., 1968; Brown, 1974; Morris et al., 1983; Nelson and Shetty, 1986.

‖ References: Grabner, 1987; Hostetler and Matsuzawa, 1981; Hostetler, 1984; Hostetler et al., 1988.

¶ Reference: Leo et al., 1971.

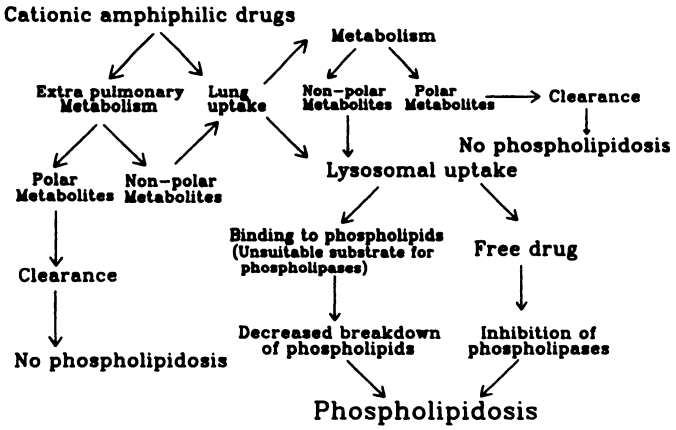

FIG. 4. Proposed mechanism of drug-induced phospholipidosis in vivo. Reproduced with permission from Joshi and Mehendale (1989).

because of the strong correlation that exists between binding to phospholipids and phospholipidosis.

The general picture of the mechanism seems to be that CADs interact with phospholipids and interfere with their metabolism at similar concentrations and with a similar potency. At least with aminoglycoside antibiotics, it was reported recently that changes in the charge on the polar side chain of the phospholipid by a drug is involved in the inactivation of phospholipase and perhaps phospholipidosis (Mingeot-Leclercq et al., 1990a,b). Although there seems to be a definitive mechanistic link between these two major effects of CADs, a finer definition of the relative involvement of either mechanism, or both mechanisms, for any particular phospholipidosis-inducing CAD needs additional experimental scrutiny.

IV. Cationic Amphiphilic Drugs, Phospholipid Metabolism, and Regulation of Cell Function

A. Cationic Amphiphilic Drugs, Phospholipid Metabolism, and Generation of Second Messengers

Understanding signal transduction pathways is important because several phospholipidosis-inducing CADs are inhibitors of phospholipases (Couturier et al., 1984; Jeng and Blumberg, 1989). Furthermore, CAD-membrane interactions may bring about changes in receptor-mediated events (Kanaho et al., 1981; Ondrias et al., 1983; Kubo et al., 1986) either directly or indirectly through altered lipid metabolism. The importance of various kinds of lipids and lipid by-products in the regulation of cell function and growth has been increasingly recognized during the last two decades. Of particular importance in this regard are phosphatidylinositol, diacylglycerol, arachidonic acid, prostaglandins, platelet-activating factors, and leukotrienes (Famulski and Carafoli, 1984; Farese, 1988; Kaczmarek, 1988; Stacey, 1988; McManus and Deavers, 1989; Tada and Kadoma, 1989). Any change in the metabolism of membrane phospholipids directly or indirectly influences one or more of the important components of the phospholipid-signaling pathway (Surewicz and Epand, 1986; Farese, 1988). A few examples may be considered in this connection. Perturbation of these pathways can affect protein phosphorylation in many

different ways (Blackmore, 1988; Stull et al., 1988; Colbran et al., 1989; Taylor, 1989).

The family of enzymes catalyzing phosphorylation are the protein kinases. These enzymes are a structurally diverse group of proteins differing in size, subunit structure, localization, mechanism of action, and substrate specificity (Nishizuka, 1984; Blackshear, 1988; Taylor, 1989; Colbran et al., 1989). Protein kinases are regulated by endogenous regulatory substances. For example, protein kinase C is activated with diacylglycerol liberated by breakdown of phospholipids (Farese, 1988). Generation of diacylglycerol by phospholipase C activation stimulates protein kinase C and protein phosphorylation directly (Nishizuka, 1984; Housey et al., 1988; Jeng and Blumberg, 1989; Kikkawa et al., 1989; Ogita et al., 1989). Short- and long-term functional and structural changes are elicited in the cell (Nishizuka, 1984; Blackmore, 1988; Kaczmarek, 1988; Stacey, 1988; Tada and Kadoma, 1989) by protein kinase C and consequent protein phosphorylation.

Phospholipases participate in an enzymatic cascade that generates highly active lipids or transduction signals (Champe and Harvey, 1988; Farese, 1988). For example, phospholipase A_2 releases arachidonate, a precursor of prostaglandins, and phospholipase C participates in the phosphoinositide cascade (Stryer, 1988). Second messengers generated by the breakdown of phosphatidylinositol phosphates are diacylglycerol and inositol trisphosphate. Most of the effects of diacylglycerol and inositol trisphosphate are synergistic (Nishizuka, 1984; Stacey, 1988; Kikkawa et al., 1989; Tada and Kadoma, 1989). Whether, and to what extent, CADs influence this cascade of events has not been investigated in detail. Chlorpromazine is one example of a CAD known to be a potent inhibitor of phospholipases (Hostetler and Matsuzawa, 1981; Beckman et al., 1982; Hostetler, 1984; Kubo and Hostetler, 1985; Shaikh et al., 1987). Additionally, chlorpromazine is a powerful inhibitor of protein kinase C (Giedroc et al., 1985; Opstvedt et al., 1986; Jeng and Blumberg, 1989). The question is whether CADs act by inhibiting phospholipase C, and thus affect diacylglycerol-mediated protein kinase C activation, or act by inositol trisphosphate-mediated calcium mobilization.

It is known that serotonin binds to its receptor and thereby mediates the activation of phospholipase C (Corbet et al., 1985; Li et al., 1988; Reinhardt, 1989). In this case, do CADs affect serotonin metabolism by their effect on phospholipase C, receptor binding, monoamine oxidase inhibition, or simply by inhibiting serotonin uptake (Mehendale, 1984; Zychlinski and Montgomery, 1985a,b; Trouve and Nahas, 1987; Li et al., 1988)? Clearly, a myriad of effects can be envisioned.

Much work is needed before predominant and critical interactions can be identified. One must consider mutually antagonizing effects as well as potentiating effects before the predominant and telling effects can be prioritized. Hypothetical relationships, such as effects of CADs on membrane lipids and inhibition of phospholipases occurring at higher than therapeutic levels, can be envisioned as well. Proposed pathways of the involvement of CADs at various stages of the phospholipid-signaling system are illustrated in fig. 5.

The stimulus that activates protein kinase C, and thus protein phosphorylation, also leads to enhanced cell proliferation. Suppression of such events may be helpful in reducing the incidence of normal cell growth and perhaps cancer. Chlorpromazine has been postulated to be anticarcinogenic because it is a potent inhibitor of protein kinase C, is a calmodulin antagonist, and also can inhibit phospholipases (Beckman et al., 1982; Hostetler, 1984; Nishizuka, 1984; Kubo and Hostetler, 1985; Giedroc et al., 1985; Price et al., 1985; Opstvedt et al., 1986; Shaikh et al., 1987). Inhibition of protein phosphorylation by these second messenger pathways brings about inhibition of tumor promotion.

Thus, the importance of CADs as modifiers of these signal transduction pathways is beginning to be recognized. It may not be surprising that some of the CADs are proposed for use in cell growth regulation in some diseases. Chloroquine has been proposed for the treatment of AIDS because it prevents the pH-dependent entry of the human immunodeficiency virus into lymphocytes (Kagan, 1987).

Do CADs affect cellular function and growth by inhibiting phospholipases and by reacting with phospholipid-derived second messengers? If so, the mechanistic link between these changes and phospholipidosis remains to be investigated. It has been postulated that chloroquine-induced inhibition of calmodulin-stimulated phosphodiesterase as well as Ca^{2+},Mg^{2+}-ATPase activities, resulting in shape changes in erythrocyte ghosts, is due to

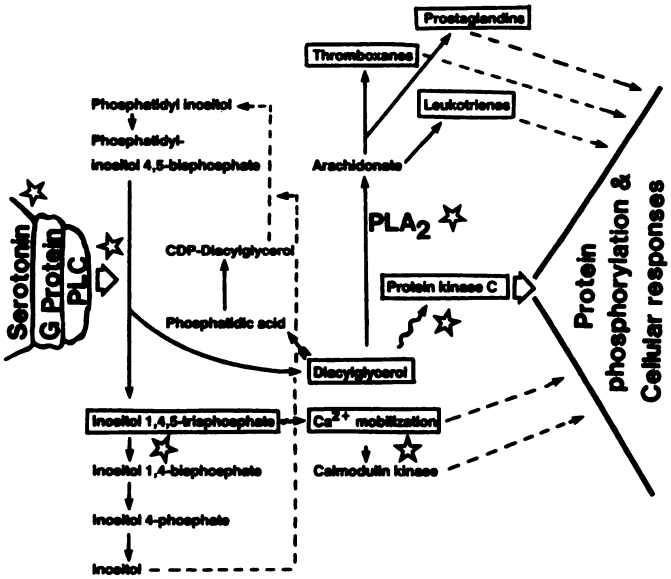

FIG. 5. Possible sites of action of CADs on the phospholipid-signaling system. PLC, phospholipase C; PLA_2, phospholipase A_2.

inhibition of phospholipases (Nagai et al., 1987). With the use of several cell culture systems, investigators have reported that chloroquine, chlorphentermine, and promethazine affect signal transduction systems (Zamora and Beck, 1986; Sauers et al., 1986; Kalisz et al., 1987; Sharp et al., 1987; Bottger et al., 1988). However, to what extent drug effects on phospholipid metabolism are involved in the alteration of signal transduction needs to be established.

Although not much has been understood of the mechanism by which some CADs affect immune functions, there are good reasons to suggest that these effects might occur (Catena, 1989). Some reports concerning CAD-mediated immune abnormalities have appeared in the literature (Jackson and Longmore, 1988; Sauers et al., 1988). Many inflammatory pulmonary disorders are thought to be initiated by deposition of immunoglobulins in alveolar capillaries. Such deposition has been reported in biopsy specimens of patients with amiodarone-induced pulmonary toxicity (Suarez et al., 1983; Joelson et al., 1984; Akoun et al., 1988). Recent reports indicate that amiodarone increases leukotriene levels and affects arachidonic acid metabolism in the lung and that these effects have been abolished by several antioxidants (Kennedy et al., 1988). One hypothesis relating to amiodarone pulmonary toxicity concerns the potential immunomodulating effects of amiodarone. However, the relationship among pulmonary toxicity, pneumonitis, and immunodysfunction has not been elucidated. Chlorphentermine-induced inhibition of immune responses are presumably due to an inhibition of phospholipases (Sauers et al., 1986; Kacew, 1987, 1988), leading to a decreased production of inositol trisphosphate and diacylglycerol from phosphoinositides. Promethazine also possesses immunosuppressive activity (Orlowski et al., 1983; Rychlik et al., 1988). However, the mechanism of its action is unknown. In our drug-phospholipid-binding studies, we observed that promethazine binds extensively to isolated lamellar bodies and phospholipid vesicles (Joshi et al., 1988, 1989). The phospholipidosis-inducing potency and phospholipase-inhibiting property of promethazine have not been investigated. Chlorpromazine and verapamil accumulate in the lung (Bakhle and Vane, 1974) and are known to inhibit antigen-induced release of slow reacting substance of anaphylaxis in the cat lung (Dell'Osa and Temple, 1986). It might be suggested that the immunosuppressive effect of promethazine is due to its effect on the phospholipid-signaling system.

From these few examples of CADs known to affect the immune responses, it could be suggested that the effects of CADs on the immune system may represent a general phenomenon rather than a drug-specific effect. Because CADs interfere with the phospholipid-signaling system in a more general way, it is likely that their effects on the immune system are through signal transduction pathways. However, this generalization needs to be carefully evaluated using various experimental approaches. Generalization will only be possible after carefully selected representative CADs are experimentally scrutinized for these effects.

B. Ion Transport Across Cell Membranes

As discussed earlier, CADs alter lipid dynamics of membranes, affect receptor function, and inhibit phospholipases. Thus, it is not surprising that they alter ion transport mechanisms in the cell and phosphorylation pathways such as oxidative phosphorylation (Zychlinski and Montgomery, 1985a). Cytosolic free Ca^{2+} modulates several calmodulin-dependent protein phosphorylation processes and, similarly, free Ca^{2+} levels are modulated by hormones, receptors, protein kinases, and feedback control by protein phosphorylation (Blackmore, 1988; Farese, 1988; Kaczmarek, 1988).

Amiodarone inhibits Na^+,K^+-ATPase and Mg^{2+}-ATPase activities in the rat lung where phospholipid storage also occurs maximally (Reasor et al., 1989) as well as in other tissues, thus affecting active ion transport phenomena and oxidative phosphorylation (Prasada Rao et al., 1986; Chatelain et al., 1989). These effects of amiodarone have been proposed to be due to its effect on lipid dynamics. Amiodarone induces cytotoxicity by increasing cytosolic free Ca^{2+} in bovine pulmonary artery endothelial cells (Powis et al., 1990). The therapeutic action of amiodarone also relates to its effect on the Ca^{2+} transport system in the heart. If amiodarone inhibits phospholipases, it should inhibit activation of protein kinase C by diacylglycerol and at the same time inhibit synthesis of inositol trisphosphate which mediates Ca^{2+} release from intracellular storage. Thus, one would expect an actual decrease in Ca^{2+} levels following amiodarone administration, unless there is a very high stimulation of influx of Ca^{2+} from the extracellular environment or Ca^{2+}-pumping mechanisms are impaired. The exact mechanism by which amiodarone modulates the calcium-signaling system is unknown.

Chloroquine, another lysosomotrophic and phospholipidotic drug, inhibits calmodulin-mediated stimulation of phosphodiesterase and Ca^{2+},Mg^{2+}-ATPase activities in red blood cells. These changes are associated with shape change in erythrocyte ghosts (Nagai et al., 1987). Alteration of oxidative phosphorylation involving mitochondrial membranes also has been reported in vivo following chlorphentermine administration (Zychlinski and Montgomery, 1985a,b, 1986). Increases in imipramine toxicity in rats by Ca^{2+} modulators which exert antagonistic effects against catecholamines and serotonin suggest a neurotransmitter-mediated enhancement of Ca^{2+} release by imipramine. Effects on oxidative phosphorylation by prolonged imipramine treatment have been proposed to be due to an alteration in the mitochondrial membrane lipid milieu and membrane stabilization after drug treatment (Katyare and Rajan, 1988).

From such examples, it can be emphasized that CADs

may react principally by two different pathways. One is by altering receptor-mediated events and the second one is by altering the lipid dynamics of membranes and phospholipases. Many more studies of the effects of CADs on ion transport are needed to understand their role in cytotoxicity mediated by calcium and other ions.

V. Conclusions

CADs share sufficient similarities in their structure even though they come from diverse pharmacological classes. One of the many general effects of CADs is that they induce a phospholipid storage disorder in various tissues and species of animals as well as in humans. The presence of lamellated inclusion bodies, and, as far as the lung is concerned, massive infiltration of macrophages, is one of the primary characteristics of their action. Phospholipidosis-inducing potency largely depends on the affinity of CADs for the particular tissue, their ability to bind to phospholipids, their inhibitory potential toward various phospholipases, and their pharmacokinetic properties. The nature of phospholipidosis and the tissue being affected are largely influenced by the structure of the drug molecule, the nature of its hydrophilic side chain, the substitution of a halogen atom, the hydrophobicity of the ring structure, the ionic environment in the cell, and the type of phospholipids present. Generally, CADs are lysosomotrophic with few exceptions. The acidic pH of lysosomes results in the ionization of these relatively basic drugs, and the drugs are either trapped in the lysosomal milieu or are bound to the membranes.

CAD-induced phospholipidosis, at least with chlorphentermine, influences respiratory function to a minimal extent. Non-respiratory or biochemical functions of the lung are perturbed to a much greater extent by CADs. Pulmonary clearance of circulating vasoactive substances is impaired by CADs and CAD-induced phospholipidosis. Although these compounds react with phospholipids and inhibit phospholipases, the precise mechanism involved in phospholipid accumulation is not clear. Evidence that CAD administration renders phospholipid an unsuitable substrate for phospholipases needs to be considered further as does CAD-associated inhibition of phospholipases in vivo and alteration of intracellular pH. Certainly, CAD-induced phospholipid storage disorder offers an important experimental model to study the relative importance of these factors in the synthesis and turnover of pulmonary phospholipids.

One should be critical when interpreting the cause of phospholipidosis in vivo, especially with regard to its relationship to phospholipid binding and phospholipase inhibition because the pharmacokinetic handling of individual CADs can be an important determinant in influencing the ability of CADs to bind to phospholipids and cause phospholipase inhibition. Thus, examples of drugs known to be bound to phospholipids and having affinity for accumulation in lung slices, as observed in in vitro experiments, may not induce pulmonary phospholipidosis because in in vivo such drug accumulation may be obviated by pulmonary metabolism and elimination.

CADs affect ion transport, immune function, tumor growth, serotonin metabolism, and several other functions in the body. Some of these effects could be correlated with the ability of CADs to interact with phospholipids and phospholipases. Extensive therapeutic use and associated side effects of CADs have generated a great deal of interest in understanding CAD-induced phospholipidosis.

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