ADVANCES IN BEHAVIORAL BIOLOGY • Volume 33

LECITHIN

Technological, Biological, and Therapeutic Aspects

Edited by
ISRAEL HANIN
and
G. BRIAN ANSELL

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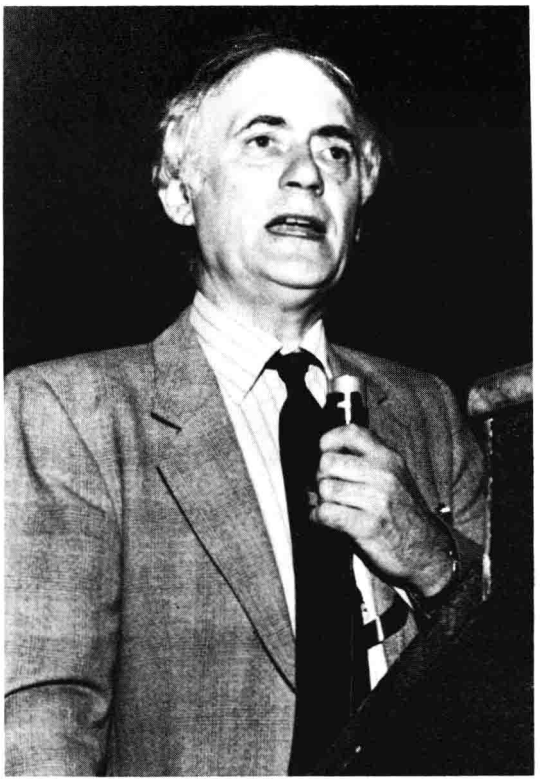

GORDON BRIAN ANSELL

1927 - 1986

DEDICATION

Brian Ansell was born in Birmingham, England on January 24, 1927. He graduated from King Edward's High School in the Edgbaston section of Birmingham. He was at Trinity College, Cambridge from 1944 to 1947 where he did the natural sciences tripos with Part I in Chemistry, Physiology, Zoology and Biochemistry and Part II in Biochemistry. The B.A. degree was awarded in 1947 and the M.A. degree in 1951. His education was interrupted from 1947 to 1949 by national service in the Royal Air Force. From 1949 to 1958 he was in Cardiff at the Neuropsychiatric Research Center in Whitchurch Hospital, where he studied for the Ph.D. under Dr. Derek Richter. In 1952 he was awarded the Ph.D. degree by the University of Wales. he then stayed on as a Senior Biochemist through 1958. Beginning in 1959 he returned to Birmingham as a lecturer in neurochemistry in the Department of Experimental Psychiatry of the Medical School. He was promoted to Senior Lecturer in 1964 and to Reader in 1967. At present this department is the Department of Pharmacology (Pre-Clinical). While at the University of Birmingham he was awarded the D.Sc. degree in 1964 in recognition of his very substantial body of experimental studies.

Brian was extremely active in two societies, The International Society for Neurochemistry and Biochemical Society. With the International Society for Neurochemistry he was a member of the

council from 1969 to 1973, then was Treasurer from 1973 to 1977, and was Chairman from 1977 to 1979. After that he was the registered agent because it is incorporated in the United Kingdom. With the Biochemical Society he also served as member of committee, member of the publications committee, chairman of the neurochemical group, archivist, and publications secretary. The latter position which was held for six years included responsibility for the Biochemical Journal and all other publications of the Biochemical Society.

For the Journal of Neurochemistry, Brian was a member of the editorial board from 1965 to 1972 and then Deputy-Chief Editor until 1976. He was involved in the organization of the international neurochemistry meeting held in Oxford in 1965 and was local organizer of a satellite meeting of the International Society for Neurochemistry on Phospholipid Metabolism in the Nervous System in 1981. Together with J.N. (Tim) Hawthorne, he produced books on phospholipids in 1964, 1973, and 1982, that were published by Elsevier in Amsterdam. These books have been very important for the development of research in the area of phospholipids and their metabolism.

The research of Brian Ansell began with the studies of proteases and amino acids. He then began studies of water soluble ethanolamine compounds including phosphoethanolamine and glycerophosphoethanolamine. These led him into the study of phospholipid turnover. He did important work in the early 1960s on plasmalogens and glycerol ethers, which we now know as the ether-linked glycerophospholipids. It was as a result of reading these papers that I applied for a post-doctoral fellowship to study with Brian. He was particularly concerned with function of the brain and the relationship of choline phospholipids with the cholinergic system. He was the first to study the choline phosphotransferase for the synthesis of choline glycerophospholipids from CDPcholine and is well known for his numerous studies dealing with choline phospholipids. During his last visit to the United States in September of 1986, he discussed his current work on the phosphodiesterase acting on glycerophosphocholine.

Sheila Spanner joined Brain Ansell's laboratory in 1959 as a technician. She completed her academic degrees through the Ph.D. degree by thesis and examination with research work done in the laboratory. I will always treasure the year that I was privileged to spend in the laboratory with Brian and Sheila form 1964-65. Numerous other students and post-doctoral fellows such as Tadeusz Chojnacki from Warsaw also have had the opportunity to learn with Brian and Sheila.

Brian had two primary hobbies. The first of these was cinema and films. He certainly would have enjoyed a period of retirement with all of the video tapes of the classic films that are available today. While I was in Birmingham I became interested in collecting coins. We were soon in the habit of checking our change each day for new dates to add to our collections.

Brian passed away in November of 1986, just six weeks after the meeting in Chicago. He is survived by his widow Edwina and two children, Caroline and Christopher. The sudden loss of Brian reminds us again to treasure our friends and loved ones while they are with us and we can enjoy them.

Lloyd A. Horrocks

PREFACE

Recently, there has been tremendous scientific interest in the role of phospholipids and particularly phosphatidylcholine (lecithin) in a variety of biological processes. These include the involvement of phosphatidylcholine in biological membranes, as a component of plasma lipoprotein, as a transporter of choline in the body, and also as a "reserve", and possibly only source of unesterified choline. Moreover, numerous clinical studies have recently been conducted, to evaluate the possible uses of externally supplied lecithin in the treatment of some intractible neuropsychiatric disorders (e.g. tardive dyskinesia, Alzheimer's disease, etc.) and other conditions (e.g. hypercholesteremia).

Results to date are encouraging, yet equivocal. This is due, in part, to the fact that the field of phospholipid methodology is highly complex. There is much confusion in the literature, and many ambiguities still remain in the interpretation of experimental findings. This is particularly so for phenomena involving phospholipid function in the central nervous system.

This book incorporates the proceedings of the Fourth International Colloquium on Lecithin, which took place in Chicago, Illinois, USA, on September 15-17, 1986. The purpose of this colloquium was to review, in a comprehensive manner, basic principles as well as current information about the technology, biochemistry, physiology and therapeutic potential of lecithin. Over 108 individuals from all over the world participated in the sessions. The meeting was subdivided into oral presentations and panel discussions (see Table of Contents). It was sponsored and financed by the Lucas Meyer GmbH (Hamburg), for which the editors are most grateful.

The scientifically stimulating and exciting atmosphere generated during the colloquium was, subsequently, marred by the death of Dr. G. Brian Ansell, less than two months after this meeting, as the result of a heart attack on November 20th, 1986. Brian had had a prior heart attack about a year earlier, but he felt well enough to continue to be involved in the planning phases of this colloquium, and he flew in to the United States from England, in order to participate in the colloquium. During the meeting he participated actively in all the proceedings and was, as always, his usual witty, alert and effusive self. Most of his friends and colleagues who saw Brian for the last time at this meeting will therefore remember him as such. I, personally, feel a keen sense of loss. Dr. Lloyd Horrocks, in his tribute which follows, expresses so effectively the feelings that I am sure many of Brian's friends and scientific colleagues felt when they found out about his sudden death.

Dr. G. Brian Ansell has been a lifelong contributor to our understanding of many aspects of phospholipid metabolism and function. This was the last major undertaking which he was involved in before his death. He worked so diligently in assuring that all aspects of the planning of this conference, and bringing it to fruition, were achieved. Furthermore, the success of this conference was extremely important to him. It therefore is befitting that this book should be dedicated to his memory. He will be both remembered, and missed.

Israel Hanin

Chicago, IL USA

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OVERVIEW ON PHOSPHOLIPIDS: CHEMISTRY, NOMENCLATURE AND ANALYTICAL METHODOLOGY

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After decades in which phospholipids were considered as inconvenient, impure mixtures of intractable materials, claiming the attention of only a few devotees (Klenk, Levene) the era commencing in about 1950 has been one of intense fundamental research on their nature, metabolism and function in animals, micro-organisms and plants.

New methods of synthesis pioneered by Baer, Malkin and van Deenen were paralleled by new methods of analysis, first an ingenious technique using partial hydrolysis introduced by Dawson, and then a flood of chromatographic methods involving separations on columns of alumina, silica and cellulose. There is now a vast armamentarium including thin layer chromatography, gas-liquid chromatography and high performance liquid chromatography. It is likely that few if any novel phospholipids remain to be discovered and attention is now focussed on the role of phospholipids in living systems.

The recommendations for the nomenclature of phospholipids proposed in 1976 by the IUPAC-IUB Commission on Biochemical Nomenclature are rather complicated but at least there is some order now. Glycerophospholipids are derivatives of sn-glycerol 3-phosphate (e.g. 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine) and sphingophospholipids are derivatives of 4-E-sphingenine (sphingosine). Some of the nomenclature is idiosyncratic, as is that for plasmalogens and a cursory glance at the literature will disclose some "consumer resistance".

Though the size of phospholipid molecules are of the same order there are infinite, subtle modifications of the long chain fatty acids and aldehydes which must be related to their structural as well as metabolic roles. Our knowledge of the special functions of phospholipids containing alkyl, alkenyl groups or carbon-phosphorus bonds is still scanty as is a role for sphingosine. It is a curious irony that a phospholipid whose structure was established only in the early nineteen sixties, phosphatidylinositol 4,5,-bisphosphate, now has a firmly established role as an integral component of a "second messenger system". Furthermore, the hydrophilic component of the molecule serves as one messenger and the hydrophobic component another. There is, in short, a whole new era in phospholipid research before us.

MODERN TECHNIQUES FOR THE FRACTIONATION AND PURIFICATION OF PHOSPHOLIPIDS FROM BIOLOGICAL MATERIALS

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Gianfrancesco Goracci, Serena Porcellati, and Young Yeo

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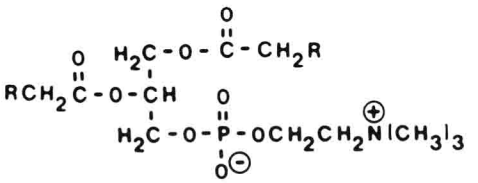
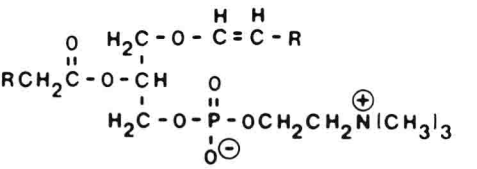
INTRODUCTION

Glycerophospholipids have important roles in biological processes. In addition to forming the hydrophobic backbone of cell membranes, glycerophospholipids also participate actively in signal transduction. A number of receptors respond to agonist-binding by activation of a phospholipase C that hydrolyzes PtdIns 4,5-P_2 to $InsP_3$ and diacylglycerols (Berridge and Irvine, 1984; Berridge et al., 1985; Berridge, 1986; Nishizuka, 1984a, b; Hirasawa and Nishizuka, 1985; Abdel-Latif et al., 1985; Akhtar and Abdel-Latif, 1986). The $InsP_3$ may increase cytosolic Ca^{2+} concentrations, thus activating a protein kinase and other reactions. The diacylglycerol stimulates protein kinase C.

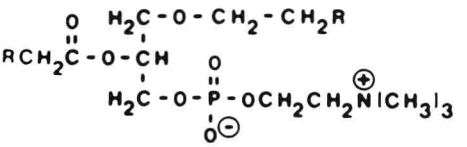
Choline glycerophospholipids may also be involved in signal transduction (Snyder, 1985). Several cell types form platelet activating factor (Lee et al., 1986; Benveniste, 1985; Oda et al., 1985; Venuti, 1985). This begins with a receptor-associated hydrolysis of 1-alkyl-2-arachidonoyl-sn-glycero-3-phosphocholine (see Fig. 1) to produce 1-alkyl-2-lyso-GroPCho and free arachidonic acid, the precursor of numerous eicosanoids (Albert and Snyder, 1983; Touqui et al., 1985; Sanchez-Crespo et al., 1985). The lyso PakCho is then acetylated at the 2-position to produce platelet activating factor (Fig. 1). This takes place in most inflammatory cells. Platelet activating factor not only activates platelets, but also stimulates neutrophils to release leukotriene B_4 (Poitevin et al., 1985). Smooth muscle has platelet activating factor receptors (Doyle et al., 1986; Yousufzai and Abdel-Latif, 1985). Platelet activating factor is involved in anaphylactic shock, histamine release, acute inflammation, graft rejection, and gastrointestinal ulcerations (Bourgain et al., 1985; Orlov et al., 1985; Hayashi et al., 1985; Saeki et al., 1985; Feuerstein et al., 1985; Hwang et al., 1986; Braquet et al., 1985). In the perfused rat liver, platelet activating factor mediates glycogenolysis and vasoconstriction (Buxton et al., 1986a, b). The reactions and enzymes of ether-linked glycerophospholipids have been reviewed (Snyder, 1985; Hanahan, 1986).



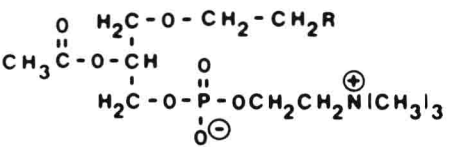
1,2-diacyl-sn-Gro-3-PCho
(phosphatidylcholine, PtdCho)



1-alk-1'-enyl-2-acyl-sn-Gro-3-PCho
(plasmenylcholine, PlsCho)



1-alkyl-2-acyl-sn-Gro-3-PCho
(alkylacyl-GroPCho, PakCho)



1-alkyl-2-acetyl-sn-Gro-3-PCho
(platelet activating factor)

Fig. 1. Nomenclature of choline glycerophospholipids. Several systems of nomenclature are used for the choline glycerophospholipids. The 1,2-diacyl-sn-Gro-3-PCho (utilizing the abbreviations suggested by the IUB-IUPAC) has the common name phosphatidylcholine. The latter should be used only for the diacyl type according to the recommendations of the IUB-IUPAC, but the term phosphatidylcholine and the abbreviation PC are often used for the mixture of all three types of choline glycerophospholipids. PC is ambiguous because it can mean phosphocholine (PCho) or phosphatidylcholine (PtdCho).

The common name for 1-alk-1'-enyl-2-acyl-sn-Gro-3-PCho is choline plasmalogen. The recommended name, plasmenylcholine, is not yet used very often but can be useful. We suggest the abbreviation PlsCho for plasmenylcholine. No common name exists for 1-alkyl-2-acyl-sn-Gro-3-PCho.

The nomenclature commission suggested plasmanylcholine but in our opinion this is too easily confused with plasmenylcholine. Without coining a new term, the shortest distinctive name is alkylacyl-GroPCho. We suggest the abbreviation PakCho to indicate a choline glycerophospholipid with an alkyl group. This is consistent with the name "phosphalkanylcholine" proposed by P. Karlson of Marburg in 1974 (letter to members of the working group on the nomenclature of lipids).

Choline plasmalogens (Fig. 1) are another source for the receptor-mediated release of arachidonic acid and subsequent formation of eicosanoids (Horrocks et al., 1986a, b). Platelet aggregation by thrombin includes the metabolism of arachidonic acid from choline and ethanolamine plasmalogens (Fig. 2). Human platelets were prelabeled with [^{3}H]arachidonic acid, then stimulated with a low dose of thrombin. During the first 20 sec, the radioactivity in the PlsCho increased 25%, then decreased. Between 20 sec and 3 min, the radioactivity in PlsEtn doubled.

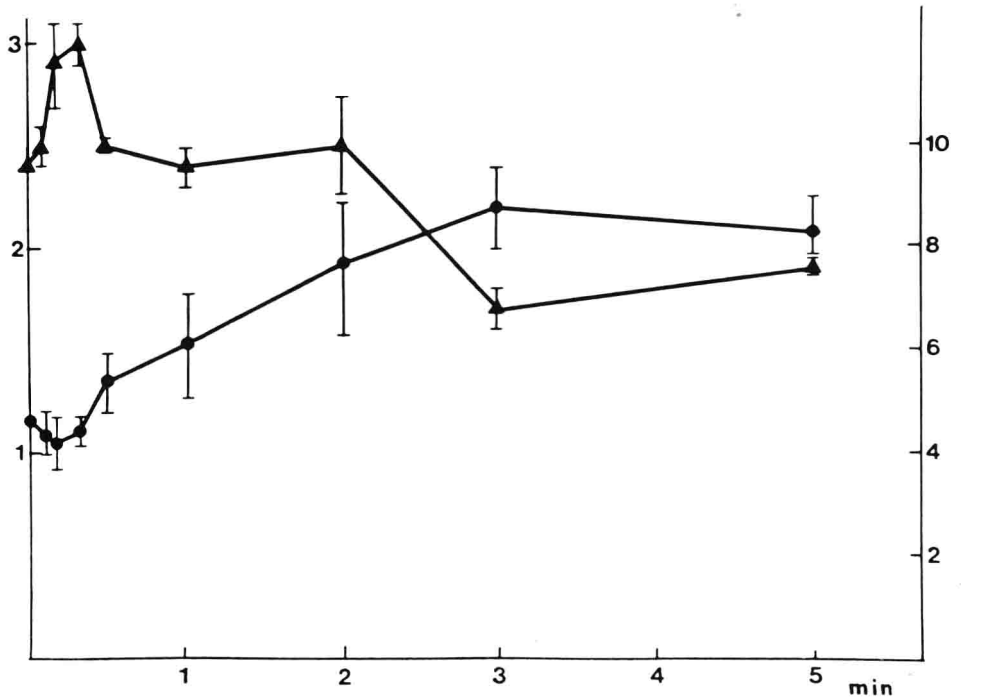


Fig. 2. Effect of stimulation with thrombin on the radioactivity of plasmalogens in platelets prelabeled with arachidonic acid. Human platelets were prelabeled with [^{3}H]arachidonic acid (Porcellati et al., 1986) and washed (Kinlough-Rathbone et al., 1983) except that 1 µM PGI_2 was also added to the medium. The platelet concentration was adjusted to $2 \cdot 10^5$ cells·ml^{-1} in Tyrode-albumin buffer.

Platelets, 0.5 ml, were stimulated with thrombin, 0.5 U·ml^{-1}, and the distribution of the radioactivity was determined at the indicated times. The filled triangles and left-hand scale are for PlsCho and the filled circles and right-hand scale are for PlsEtn, percent radioactivity.

The changes in levels of radioactivity indicate hydrolysis and reacylation of the plasmalogens. The ether-linked glycerophospholipids incorporate arachidonic acid at the 2-position by transarachidonoylation from the 2-position of PtdCho (Malone et al., 1985; Sugiura and Waku, 1985; McKean and Silver, 1985; Sugiura et al., 1985; Robinson and Snyder, 1985; Colard et al., 1986). The transfer is energy independent. The role of plasmalogens as important sources of free arachidonic acid is often overlooked because the active metabolism is masked by the transarachidonoylation. The ether-linked lysoglycerophospholipids may also be involved in secretory responses such as glucose-induced insulin secretion (Metz, 1986). The very early hydrolysis of the choline plasmalogens may be linked with the entry of external Ca^{2+}.

A massive influx of extracellular Ca^{2+} into cells is found immediately after trauma of the spinal cord (Stokes et al., 1983; Stokes et al., 1984; Stokes et al., 1985; Young and Flamm, 1982; Young and Koreh, 1986). The largest change in the glycerophospholipids at that time is the loss of 10-18% of the PlsEtn which comprises more than one-third of the membrane phospholipids (Demediuk et al., 1985). Polyphosphoinositides were not examined.

Muscle tissues contain relatively large amounts of both choline and ethanolamine plasmalogens (Horrocks, 1972; Horrocks and Sharma, 1982; Gross, 1985; Arthur et al., 1985; Kostetskii and Sergeyuk, 1985). Values for bovine muscles are 19% PlsCho and 21% PlsEtn in the total phospholipids. Electrical stimulation of the tissue caused a loss of 18-20% of both plasmalogens with no significant change in any other glycerophospholipid. Polyphosphoinositides were not examined in this study. The stimulation of the beef muscle may activate phospholipases associated with receptors. A phospholipase A_2 with specificity for plasmalogens has been detected in heart muscle and purified from platelets (Wolf and Gross, 1985; Loeb and Gross, 1986).

Glycerophospholipids with alkyl groups have antitumor activities (Andreesen et al., 1978, 1982; Berdel et al., 1981). Synthetic derivatives of 2-lyso PakCho cause the accumulation of alkyl-containing glycerophospholipids in tumor cells because they are deficient in the alkyl-cleavage enzyme. Normal cells metabolize the compounds and are not affected. The N-acyl derivatives of EtnGpl accumulate in infarcted canine myocardium and cerebrum (Natarajan, 1981; Natarajan et al., 1980) and in degenerating chick embryo tissues in fertilized hens' eggs (Kara et al., 1986). A tumor-selective cytolytic phospholipid preparation from the latter source was fractionated. The active component was the N-palmitoyl derivative of PakEtn (Kara et al., 1986). It is non-toxic for normal cells but inhibits DNA synthesis by malignant cells at nanomolar concentrations.

The differences in metabolism of glycerophospholipids with acyl, alkenyl, or alkyl groups at the 1-position indicate a requirement for analytical methods to separate all classes. Marked metabolic differences are also found for different molecular species within specific classes. For example, the turnover of PlsCho, PakCho, and PtdCho with 18:0 side-chains at the 1-position is much slower than the turnover of the compounds differing only in having an 18:1 side-chain (Horrocks et al., 1986a, b). In addition, the polyphosphoinositides, free fatty acids, and diacylglycerols should also be extracted and quantitated. The accepted methods and their limitations are reviewed in much greater detail in a book from the Neuromethods series (Boulton et al., 1987).

Most studies of glycerophospholipids and related products associated with signal transduction have focused on polyphosphoinositides and have used acidic extraction solvents. The addition of HCl to solvents seems to be necessary for removal of the polyphosphoinositides from interactions with the proteins (Hauser and Eichberg, 1973). Unfortunately, these strongly acidic solvents also produce large amounts of artifactual diacylglycerols and free fatty acids (Table 1). In addition, all of the plasmalogens are hydrolyzed to produce long-chain aldehydes and lysoglycerophospholipids.

Table 1. Effects of hydrochloric acid on lipid extraction from rat brain.

	Neutral	Acidified
	nmol lipid per g wet weight, mean ± S.E.M. (n=6)	
PtdIns 4-P	137 ± 5	228 ± 13
PtdIns 4,5-P_2	177 ± 7	572 ± 22
Free fatty acid	161 ± 12	1100 ± 137
Diacylglycerols	312 ± 23	714 ± 94

Rats were killed by microwave irradiation. The brains were extracted with 2:1 chloroform/methanol (Folch et al., 1957) or with 0.5 M HCl also included (T.S. Reddy, L.A. Horrocks, and N.G. Bazan, unpublished).