evaporator. The resulting white crystalline solid was recrystallized from 1-butanol. Drying *in vacuo* at 40° yielded 6 g (95.1%) of product, mp 215° dec. *Anal.* ($C_{11}H_{11}N_3O_3$) C, H, N.

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Isolation and Renin-Inhibitory Activity of Phosphoglyceride from Shark Kidney

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We report on the isolation, fatty acid composition, and renin-inhibitory activity of a chromatographically homogeneous phosphoglyceride from the kidney of the Mako shark, *Isurus oxyrinchus*. Kidney phosphoglyceride from the shark, a lower vertebrate fish, was found to be similar in structure and in activity to mammal (hog) kidney phosphoglyceride; this finding may have some bearing on the question of a possible physiologic role for phosphoglyceride as natural renin inhibitors,[1-5] since renin has been reported[6,7] not to be present in the kidneys of sharks.

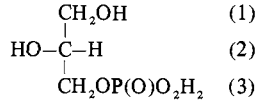
1-Acyl-*sn*-glycerolyso-3-phosphatides† of naturally occurring phosphoglycerides have been reported to inhibit the enzyme renin *in vitro* and the response of injected renin *in vivo*.[2-4] This type of bioactive lysophosphoglyceride, termed *renin inhibitor*,[5] has been shown to be produced *in vitro* by phospholipase A from parent phosphoglyceride, which has been termed *renin preinhibitor*.[5] Parent renin preinhibitor itself has some renin-inhibitory activity *in vitro*[2] but is not nearly as potent as its lysophosphoglyceride; *in vivo*, enzymic hydrolysis of renin preinhibitor to renin inhibitor could be anticipated, but experimental proof of systemic occurrence and distribution of inhibitor-type lysophosphoglyceride is presently lacking. Renin preinhibitor has been isolated from dog and hog kidney[1-3] and also from other organs (heart, spleen, liver) of the rat and dog.[9] Its presence has been demonstrated in plasma and erythrocytes of the rat, dog, and anepheric patients.[9-11] Canine renin preinhibitor reduces plasma reinin activity and blood pressure to near normal levels in both acute and chronic renal hypertensive rats and is reported to have no observed effect on blood pressure of normal rats in spite of causing reduction of plasma renin activity.[4]

Renin preinhibitor from dog and hog kidney is characterized by a high percentage of esterified polyunsaturated fatty acid identified mainly as arachidonate, but the composition and positional distribution of the fatty acids of each individual phosphoglyceride‡ molecule has not been established. Experimental evidence has confirmed that these naturally occurring lipids are phosphatidylethanolamines[5,9,13,14] or related lipids.[5] Identification of renin inhibitor as lysophosphoglyceride also has been indicated by studies[15] with synthetic dilinolenyl phosphatidylethanolamine, which, as the lysophosphoglyceride, was found to have activity nearly comparable to that of natural renin inhibitor and, by inhibition of renin with a number of synthetic lysophosphatidylethanolamines, 2-desoxylysophosphatidylenthanolamines,[13] tridesoxylysophosphatidylethanolamines,[16] and related derivatives.[17]

Isolation. In these studies 1.88 g of crude phosphoglyceride (I) was isolated from 1 kg of Mako shark kidney. Crude I, as analyzed by tlc, consisted of two major and three minor phospholipid components; I contained 3.73% phosphorus and 2.11% nitrogen. Column chromatography of this mixture separated a major homogeneous (tlc) highly unsaturated phosphoglyceride II. The infrared and pmr spectra of II were consistent with those reported[18,19] for naturally occurring phosphoglyceride molecules. The pmr spectrum of II included a broad absorption at δ 8.24-8.68 characteristic of acidic phosphatidylethanolamine polar head protons [-(O)P(O*H*)$OCH_2CH_2NH_2$ and/or -(O)$P(O^-)$-$OCH_2CH_2NH_3^+$].[19] The presence of a CH_2N signal at δ 3.26 further indicated that II is predominantly multispecies phosphatidylethanolamine, since this signal is absent in α-amino acid phosphoglycerides, such as phosphatidylserines; the absence of a signal (δ 3.4)[19] characteristic of trimethylammonium protons revealed that II was not phosphatidylcholine. Chemical shifts of glyceryl, polar head methylene, and saturated and unsaturated fatty acyl chain protons were in accord with literature values;[19] seven distinct absorptions due to protons of esterified polyunsaturated fatty acid were readily assigned when the 100-MHz spectrum of II was compared with spectra of methyl arachidonate, arachidonic acid, and a number of other arachidonyl derivatives.§

The methyl ester compositions of the fatty acids obtained upon methanolysis of II are presented in Table I.

†Stereospecific numbering (*sn*)[8] is used to designate the absolute configuration of naturally occurring phosphoglycerides as derivatives of *sn*-glycero-3-phosphoric acid.



(1)
(2)
(3)

‡Effective analytical methods are not available for complete separation of intact individual molecules within classes of naturally occurring phosphoglycerides.[12] Therefore, the term renin preinhibitor, when used to describe natural phosphoglyceride isolated from different animal or organ sources, does not necessarily mean phosphoglyceride of identical multispecies but can be considered as homogenous fractions usually indistinguishable by thin-layer chromatography and having similar biological activities.[10]

§J. G. Turcotte, unpublished data.

Table I. Methyl Esters of Fatty Acids Obtained from Methanolysis of Shark Kidney Phosphoglyceride II

Fatty acid methyl ester	Weight, %
15.5[a]	8.65
16:0[b]	11.39
16:1	2.25
17.5[a]	1.80
18:0	19.28
18:1	9.62
20:1	1.14
21.6[a]	1.26
20:4[c]	26.34
20:5	9.78
22:5	1.64
22:6	6.85
	100.0

[a]Carbon number value determined on a DEGS column relative to stearic acid (18:0). [b]Normal carbon chain length:number of double bonds. [c]Several cis 20:4 naturally occurring nonconjugated fatty acids are known;[20] arachidonic acid (20:4, 5c, 8c, 11c, 14c) is abundant in animal tissue.

Table II. Renin-Inhibitory Activity of Shark Kidney Phosphoglyceride

Assay no.	Sample	Amount, mg	Angiotensin produced, ng[b]	% inhibition
1	Hog kidney lipid[a]	5.8	105.3	20.7
2	Hog kidney lipid[a]	5.8	90.6	36.6
1	Shark kidney lipid I	3.3	89.0	33.0
2	Shark kidney lipid I	3.3	93.7	34.4
2	Shark kidney lipid II	1.3	93.7	34.4
2	Shark kidney lipid II	3.3	62.3	56.4
1	Control		132.8	
2	Control		142.8	

[a]Purity comparable to shark kidney lipid I. [b]See Experimental Section.

The data show that II contained a large amount of polyunsaturated esterified fatty acid accounting for 44.6% of the total methyl esters. Arachidonic acid (20:4, 26%), an essential fatty acid, is the major polyunsaturated component of II and is also reported to comprise the highest percentage of the total fatty acid of preinhibitor phosphoglyceride isolated from dog (38%)[1] and hog kidney (25%).[13,14] The presence of 20:4, 20:5, 22:5, and 22:6 esterified fatty acid in shark kidney preinhibitor phosphoglyceride is to be expected, since this degree of unsaturation is common to fish oil fatty acid.[20]

Results and Discussion

Bioassay results of the effect of I and II on renin generation of angiotensin from dog renin substrate are presented in Table II. Under the assay conditions employed, shark phosphoglyceride I and II exhibited renin-inhibitory activity at least comparable to that of hog kidney phosphoglyceride. The high activity of I and II, as well as that of hog and dog kidney phosphoglyceride, may be associated in part with the methylene interrupted *cis*-polyethenoid esterified acids of certain molecular species of lysophosphoglycerides; results of studies with dog kidney renin preinhibitor,[1,2] synthetic phosphatidylethanolamines,[15] and related analogs appear to support this point of view, whereas other studies[13,14] apparently do not.

Although the presence of renin-inhibitory phosphoglyceride in mammals appears to be well established, the possibility of a physiologic role for this lipid or derived renin inhibitor is controversial, as are many aspects of the renin-angiotensin-aldosterone system itself. Determination of whether or not lysophospho- or phosphoglycerides do have enzyme regulatory roles is important, since the renin-angiotensin-aldosterone system is identified in man with a number of known or suspected physiologic and pathophysiologic processes. Convincing experimental evidence by Nishimura, *et al.*,[6] who demonstrated by enzymologic and histologic studies that renin was not present in the kidneys of four species of shark they investigated, suggested that examination of the kidneys of these lower vertebrates might reveal information about the possible physiologic role(s) of renin inhibitory phosphoglycerides and lysophosphoglycerides. In view of our results, if phosphoglyceride at least closely related in structure to dog and hog renin preinhibitor was *not* found to be present in shark kidney, its absence then possibly could be considered as evidence that this type of complex lipid may have a physiologic role in the renin-angiotensin system of higher animals having such characteristic lipid. The presence of renin-inhibitory phosphoglyceride in shark kidney, however, should not necessarily occlude such speculation, since phosphoglycerides occur in all body tissues and systems and in recent years have been shown to have diverse and unsuspected physiologic functions. Therefore, the action of certain species of lysophosphoglyceride on a system such as the renin-angiotensin system, which is of uncertain pathophysiologic involvement itself, may be very difficult to determine experimentally, particularly in relationship to such diseases as essential hypertension.

The apparent similarities in chemical type and in renin-inhibitory activity of shark kidney phosphoglyceride to that obtained from dog and hog kidney indicate that naturally occurring renin-preinhibitor phosphoglycerides from the kidney of several different animal species apparently are not uniquely different in molecular structure; however, these kidney lipids appear to differ from many other tissue phosphoglycerides by virtue of a relatively high content of esterified polyunsaturated fatty acid and possibly the positional distribution of such acid.[20,21,22] These observations and others thus far reported on structural features of natural phosphoglycerides and lysophosphoglycerides[5,10,12-14] that inhibit renin suggest that activity of individual or mixed molecular species of naturally occurring lysophosphoglycerides are dependent on respective singular and/or aggregate physiocochemical properties, such as solubility, micelle formation and charge, and surfactant properties, etc. Variations in such properties are known to be markedly affected by differences in length and degree and position of unsaturation of constituent fatty chains in intact phosphoglycerides.[23]

Experimental Section

Whole kidneys of intact male and female Mako sharks dead from 3–7 hr were removed, frozen (Dry Ice), and subsequently stored in a freezer (−30°) until work-up. Silica gel HF 254 (Brinkmann) was used for tlc analyses; column chromatographic purifications were made with Silicar CC-7 (Mallinckrodt). Glc analyses were determined with Hewlett-Packard Models 700 and 5750 gas chromatographs. PtO_2 was purchased from the Matheson Co., Inc., and BF_3–methanol from Supelco, Inc. Ir spectra (neat) were determined with a Beckman IR-8 spectrophotometer and the pmr spectra with Varian A-60 and Jeol JNMPS-100 high-resolution nmr spectrometers using $CDCl_3(Me_4Si)$ as solvent. Elemental analysis was performed by Micro-Analysis, Inc., Marshallton, Wilmington, Del.

Kidneys (1 kg) from the Mako shark, *Isurus oxyrinchus*, were processed essentially by the method of Sen, *et al.*,[1] to obtain 1.88 g of crude phospholipid I before further purification by column

chromatography. Thin-layer chromatograms [silica, $CHCl_3$:-MeOH:H_2O (95:36:6)] of I revealed at least five components (R_f 0.11, 0.24, 0.36, 0.64, 0.75) with major spots at R_f 0.24 and 0.36; all components gave positive color tests (ammonium molybdate-stannous chloride) for phosphorus. Elemental analysis of multicomponent I showed it to contain phosphorus (3.73%) and nitrogen (2.11%). Chromatography of 500 mg of I on a silicic acid column (0.5 × 30 cm) with $CHCl_3$:MeOH (4:1) as the eluent gave 81 mg of a homogeneous fraction II, R_f 0.38, which gave positive tests for phosphorus (ammonium molybdate-stannous chloride), unsaturation (fluorescein-bromine), and amine (ninhydrin). The R_f value of II was identical in two tlc systems with the main ninhydrin spot of crude phosphoglyceride obtained from hog kidney and with commercial bovine phosphatidylethanolamine standard.

The ir spectrum (neat) of II included bands at 3000, 1626 (C=C), 1709 (C=O), 1212 (P=O), 1053 (POC), and 900 cm^{-1} (POH) and several absorptions characteristic of polar head NH and OH stretching and deformation vibrations of phosphatidylethanolamines:[24] pmr (100 MHz) δ 0.96 (CH_3), 1.35 (CH_2), 1.80 (CH=$CHCH_2CH_2CH_2CO$), 2.14 (CH=$CHCH_2$), 2.45 (CH_2CO), 2.96 (CH=$CHCH_2CH$=CH), 3.26 (CH_2N), 3.87–4.75 (glyceryl protons, CH_2OP), 5.54 (CH=CH), and 8.24–8.68 [(O)P(O*H*)$OCH_2CH_2NH_2$ and/or (O)$P(O^-)OCH_2CH_2NH_3^+$].

Several milligrams of phospholipid II were dissolved in 3 ml of methanol–benzene (1:1), 1 ml of methanolic 0.5 *N* KOH was added, and the mixture was heated (100°) for 5 min in a Teflon-lined screw-cap centrifuge tube flushed under N_2; after cooling, 2 ml of 12% BF_3–methanol[25] then was added followed by heating (100°) under the same conditions for another 5 min. Tlc of the lipid resulting from work-up of the organic phase[25] confirmed the presence of methyl esters as the major species (*ca.* 95%).

Qualitative and quantitative analysis of the fatty acid methyl esters was performed using flame-ionization gas–liquid chromatography. The relative retention times of the methyl esters of the natural sample, before and after hydrogenation (PtO_2–Adam's catalyst, MeOH, 1 atm, 1 hr), were compared with several authentic methyl ester standards on polar (DEGS) and nonpolar (OV-1) columns. Lipids I and II were treated with phospholipase A[1,2] and the corresponding lysophosphatides were incubated with dog renin in the presence of dog renin substrate; the amounts of angiotensin formed (Table II) were assayed in the pentolinium-treated vagotomized rat.[1,2]

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Absolute Configuration by Asymmetric Synthesis of (+)-1-(4-Acetamidophenoxy)-3-(isopropylamino)-propan-2-ol (Practolol)

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In recent years many substances based on the general structure 1-aryloxy-3-alkylaminopropan-2-ol (**1**) have been prepared in different laboratories in a search for compounds with β-adrenergic receptor blocking activity. To date, it has been found that the (−) isomers are the most effective in antagonizing the effects of isoproterenol and it has been of interest to relate the absolute configuration of these compounds to that of the natural agonists, epinephrine and norepinephrine. With this objective, Dukes and Smith have related (+)-propranolol (**1**, Ar = 1-naphthyl, R = isopropyl) to (+)-lactic acid and hence shown it to have the *R* configuration.[1] The configuration of other β-adrenergic receptor blocking substances, including the two enantiomers of practolol, were then in turn related to (*R*)-propranolol by Horeau's method of partial asymmetric synthesis, and it was thus inferred that all the active (−) isomers had the same *S* configuration, stereochemically equivalent to (*R*)-(−)-epinephrine.

During the course of our work on other cardiovascular drugs, we obtained (*R*)-(−)-1-(4-acetamidophenoxy)-2,3-epoxypropane (**6**) and the reaction of this substance with isopropylamine offered a convenient opportunity to prepare an isomer of practolol (**7**) with a known absolute configuration. The compound obtained showed a positive rotation both as the free base and as the hydrochloride salt and thus by direct evidence (+)-practolol was shown to have