Vol. 99, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 16, 1981 Pages 183-188

POTENT PLATELET STIMULATING ACTIVITY OF ENANTIOMERS OF ACETYL GLYCERYL ETHER PHOSPHORYLCHOLINE AND ITS METHOXY ANALOGUES

Donald J. Hanahan[+], P. G. Munder[*], Kiyoshi Satouchi[+],
Linda McManus[†], and R. Neal Pinckard[†]
Departments of Biochemistry[+] and Pathology[†]
The University of Texas Health Science Center
San Antonio, Texas (USA)
The Max-Planck-Institut für Immunobiologie[*]
Freiburg, West Germany

Received January 26,1981

SUMMARY: Surprisingly, the sn-1 configuration of 1-0-hexadecyl-2-acetyl-glycerylphosphorylcholine showed significant activity, 3.22 x 10^{-9} M, when compared to the sn-3 enantiomer, 2.92 x 10^{-10} M and a racemic mixture with a value of 7.2 x 10^{-10} M. A methoxy substitution at the C-1 or C-2 position of octadecyl glycerylphosphorylcholine gave a derivative with high biological activity for stimulating serotonin release from rabbit platelets. A 1-0-dodecyl-2-methoxy analogue showed very low activity; also, a comparable series of 0-benzyl derivatives were inactive. Examination of 1-0-hexadecyl, 1-0-octadecyl- or 1-0-dodecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine showed that the hexadecyl compound had three times the biological activity of the octadecyl and five times that of the dodecyl.

The isolation and characterization of acetyl glyceryl ether phosphoryl-choline (AGEPC) as the platelet activating factor released from rabbit basophils into the plasma of rabbits during IgE anaphylaxis (1,2,3) has stimulated great interest particularly as regards the manner in which this compound exerts its effect(s) on cells. The potent biological activity of AGEPC on rabbit and human platelets (1,4) naturally has led to considerable speculation on the relation of specific chemical groupings on this molecule to its reactivity. To this end, we have examined the stimulatory effect of analogues of AGEPC on rabbit platelets; for example, in which a methoxy or a benzyl group has been substituted for the acetyl moiety, in which the alkyl chain length has been varied, and in addition we have examined the influence of the stereochemical configuration about the C-2 position by comparing the activity of the sn-1 and sn-3 enantiomers of 1-0-hexadecyl-2-acetyl glyceryl phosphorylcholine. The results of this study provide some provocative new

0006-291X/81/050183-06$01.00/0

insights into the structural requirements for biological activity of this potent lipid chemical mediator.

Materials and Methods: AGEPC was prepared by a semi-synthetic route as described previously (1). It was separated into a "hexadecyl rich" and an "octadecyl rich" fraction by thin layer chromatography in a solvent system of methanol/water, 2:1, v/v (5). 1-O-Hexadecyl-sn-glyceryl-3-phosphorylcholine, $[\alpha]_{589}^{20^\circ}$ -4.8° ([M] = -23.98°/mole) in chloroform (C)/methanol (M), 1:1, v/v, 3-O-hexadecyl-sn-glyceryl-1-phosphorylcholine, $[\alpha]_{589}^{20^\circ}$ $+5.2^\circ$ ([M] = 25.98°/mole) in C-M, 1:1, v/v, and rac-1-O-hexadecyl-glyceryl-3-phosphorylcholine were obtained from R. Berchtold, Biochemisches Laboratorium, CH-3002, Bern, Switzerland. 1-O-Octadecyl-2-methoxy-sn-glyceryl-3-phosphorylcholine, 1-O-methoxy-2-octadecyl-sn-glyceryl-3-phosphorylcholine, 1-O-octadecyl-propanediol-3-phosphorylcholine, 1-O-octadecyl-sn-glyceryl-3-phosphorylcholine, 1-O-octadecyl-2-benzyl-sn-glyceryl-3-phosphorylcholine, 1-O-dodecyl-sn-glyceryl-3-phosphorylcholine, 1-O-dodecyl-2-methoxy-sn-glyceryl-3-phosphorylcholine, 1-O-dodecyl-2-benzyl-sn-glyceryl-3-phosphorylcholine, and 1-O-dodecyl-propanediol-3-phosphorylcholine were synthesized as outlined by Arnold, Weltzien, and Westphal (6) and Weltzien and Westphal (7). The purity of these compounds was assessed by thin layer chromatographic analysis using pre-coated silica gel plates, 250 μ, in a solvent system of chloroform/methanol/water, 65:35:6. Compounds with a free hydroxyl function (lyso) were acetylated in chloroform by incubation with acetic acid anhydride for 2 hr at 65° to 70°C. In the usual instance, the reaction was over 95% complete, and the desired product was purified by thin layer chromatography as above. Phosphorus assay of the compounds was accomplished by the method of Bartlett (8) subsequent to perchloric acid ashing of the sample.

Biological activity was measured by examination of ^{3}H-serotonin release from washed, rabbit platelets by a previously described technique (1,7). The percent serotonin released by various concentrations of AGEPC or AGEPC analogues was plotted linearly; one unit of activity was defined as the final molar concentration of the particular compound required to effect the release of 50% [^{3}H]-serotonin under standardized conditions. Desensitization procedures were conducted as described previously (1).

RESULTS AND DISCUSSION

The data presented in Table I provided some unexpected results, particularly with respect to the activities of the methoxy substituted octadecyl glyceryl phosphorylcholines, and of the enantiomeric forms of hexadecyl-2-acetyl-sn-glyceryl phosphorylcholine. In the first instance, not only did the 2-O-methoxy substituted compound have a significant biological activity, 6.07×10^{-8} M, but the 1-O-methoxy analogue possessed an even higher biological behavior, i.e., 1.56×10^{-8} M. A dodecyl analogue exhibited a comparatively low response at 4.05×10^{-6} M. In the second instance, the finding that the sn-3, sn-1, and rac forms of hexadecyl acetylglyceryl phosphorylcholine had comparably high activities, i.e., 2.9×10^{-10} M, 3.2×10^{-9} M, and 7.2×10^{-10} M, resp. was most surprising in view of the anticipated stereochemical specificity for the sn-3 configuration.

TABLE I

Influence of Modifications at the C-1 and C-2 Positions of Acetyl Glyceryl Ether Phosphorylcholine on Secretion of [^{3}H]-Serotonin from Rabbit Platelets

	Modification	Molar Activity, based on 50% ^{3}H-Serotonin Release [+]
1.	Methoxy Substitution on alkyl sn-3 GPC	
	a. 1-0-methoxy-2-octadecyl	$1.56 \pm 0.37 \times 10^{-8}$
	b. 2-0-methoxy-1-octadecyl	$6.07 \pm 1.41 \times 10^{-8}$
	c. 2-0-methoxy-1-dodecyl	$4.05 \pm 1.83 \times 10^{-6}$
2.	Alkyl chain length variation on C-1 position	
	a. Hexadecyl (pure)	$2.92 \pm 0.26 \times 10^{-10}$
	b. Octadecyl (pure)	$9.30 \pm 0.13 \times 10^{-10}$
	c. "Hexadecyl rich" [++]	$1.52 \pm 0.41 \times 10^{-10}$
	d. "Octadecyl rich" [++]	$4.10 \pm 0.20 \times 10^{-10}$
	e. AGEPC (original)	$1.80 \pm 0.3 \times 10^{-10}$
	f. Dodecyl (pure)	$1.51 \pm 0.20 \times 10^{-9}$
3.	Stereochemical	
	a. 1-0-hexadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine	$2.92 \pm 0.26 \times 10^{-10}$
	b. 3-0-hexadecyl-2-acetyl-sn-glyceryl-1-phosphorylcholine	$3.22 \pm 0.98 \times 10^{-9}$
	c. rac-hexadecyl acetylglycerylether phosphorylcholine	$7.22 \pm 1.43 \times 10^{-10}$

[+]Mean $\pm$ standard deviation of [^{3}H]serotonin secretion from six different platelet preparations.

[++]Composition (based on chain length and percent of total):
"hexadecyl rich": 16:0, 95.7; 15:0, 3.2; 17:0, 1.1; 18:0, n.d.
"octadecyl rich": 18:0, 98.0; 17.0, 1.3; 16:0, 0.5; 15:0, 0.2

On the other hand, the influence of chain length of the alkyl ether substituent on biological activity showed a more predictable pattern with the 1-0-hexadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine yielding the highest molar activity, i.e., 2.9×10^{-10} M. The corresponding 1-0-octadecyl and 1-0-dodecyl derivatives gave lower values of 9.3×10^{-10} M and 1.5×10^{-9} M, resp. This was not totally unexpected since our laboratory (5) recently had found a similar effect of the alkyl chain length on the biological activities on AGEPC and certain of its polar head group analogues.

Lastly, substitution of a benzyl group for the acetyl (carboxylic ester) moiety resulted in complete loss of biological activity as did the

TABLE II

Reactivity of Various Phosphoglycerides on 1-0-Octadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (18AcGEPC) or 1-0-Octadecyl-2-methoxy-sn-glyceryl-3-phosphorylcholine (18MeOGEPC) Desensitized Rabbit Platelets

Stimulus	Control Platelets*	Desensitized Platelets*	
		18AcGEPC	18MeOGEPC
AGEPC (original)	51.4	11.2	5.9
18AcGEPC	50.7	11.7	6.7
18MeOGEPC	55.5	13.2	6.3
1-0-Hexadecyl-2-acetyl-sn-glyceryl-3-phosphoryl-choline (sn-3 form)	52.7	11.7	8.9
3-0-Hexadecyl-2-acetyl-sn-glyceryl-1-phosphoryl-choline (sn-1 form)	64.3	14.6	6.7
α-Thrombin (0.25 units/ml)	60.5	58.1	53.2
BSA-Saline	3.4	3.8	5.8

*% [^{3}H]-serotonin secretion in 60 seconds. Platelets were desenitized to individual compounds as described elsewhere (1,9).

complete removal of the oxygen function at the 2 position, for example in the propanediol derivative. Essentially all these compounds were negative at 10^{-6} M and at a concentration greater than 10^{-5} M they caused lysis of platelets as indicated by significant lactic dehydrogenase release. Similarly, all lyso compounds, which were used for synthesis of acetylated derivatives, were inactive at 10^{-5} M.

In order to assess whether the various methoxy substituted glyceryl ether phosphorylcholines and the enantiomeric forms of hexadecyl acetyl glyceryl phosphorylcholine initiated platelet stimulus-secretion coupling by interacting with the same platelet "receptor", desensitization studies were conducted utilizing previously published procedures (1,9). Thus, platelets were desensitized specifically to either 1-0-octadecyl-2-methoxy-sn-glyceryl-3-phosphorylcholine or to 1-0-octadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine. Desensitized and control platelets then were assessed for their reactivity to several of the biologically active alkyl ether phosphoglycerides. The data shown in Table II demonstrated that platelets desensitized either to the 2-methoxy or 2-acetyl substituted 1-0-octadecyl-sn-glyceryl-3-phosphoryl-

choline also were desensitized to all other methoxy substituted and enantiomeric forms of hexadecyl acetyl glyceryl phosphorylcholine. The specificity of the platelet desensitization is shown by a normal secretory [^{3}H]-serotonin response of the desensitized platelet to stimulation with α-thrombin. Thus, these studies demonstrated that all of the biologically active phosphoglycerides tested here probably interact with the same receptor or membrane binding site required to initiate the platelet release reaction.

The evidence obtained in this study regarding the structural features of AGEPC most important to its biological activity is puzzling in one sense, but highly provocative in another. On initial appraisal of the high potency of the methoxy analogues, it would seem less than likely that deacetylation would play an important role as postulated earlier (2) in the initial phase of AGEPC action on cells. However, one cannot dismiss the possibility that removal of an acetyl group and/or utilization of lyso-glyceryletherphosphorylcholine for further metabolic processing does occur until it is proven that the methoxy derivative is not susceptible to rapid cleavage in the stimulus reaction. Further, the finding that both the sn-3 and sn-1 forms of AGEPC had high activity would argue against the usual type of hormone-like receptor sites. Recent studies on polar head group analogues of AGEPC (9) would suggest a lipid rich environment for the AGEPC interaction site on platelets. It is evident, then, that interpretation of the mode of action of AGEPC on cells will require an entirely new approach to investigation of stimulus-cell interaction.

ACKNOWLEDGEMENT

This study was supported by a grant, HL-22555-03, from the National Institute of Heart, Lung and Blood. We wish to acknowledge the excellent technical assistance of Richard Castillo and Carol Lange.

REFERENCES

1. Demopoulos, C. A., Pinckard, R. N., and Hanahan, D. J. (1979) J. Biol. Chem. 254, 9355-9358.

2. Hanahan, D. J., Demopoulos, C. A., Liehr, J., and Pinckard, R. N. (1980) J. Biol. Chem. 255, 5514-5516.

3. Pinckard, R. N., Farr, R. S., and Hanahan, D. J. (1979) J. Immunol. 123, 1847-1857.

4. McManus, L. M., Hanahan, D. J., and Pinckard, R. N. (1981) J. Clin. Invest., in press.

5. Satouchi, K., Pinckard, R. N., McManus, L., and Hanahan, D. J. (manuscript in preparation).

6. Arnold, D., Weltzien, H. U., and Westphal, O. (1967) Liebigs Ann. Chem. 709, 234-239.

7. Weltzien, H. U. and Westphal, O. (1967) Liebigs Ann. Chem. 709, 240-243.

8. Bartlett, G. (1959) J. Biol. Chem. 234, 466-468.

9. Satouchi, K., Pinckard, R. N., McManus, L. M., and Hanahan, D. J., submitted to J. Biol. Chem.