The Lancet · Saturday 15 July 1978

EICOSAPENTAENOIC ACID AND PREVENTION OF THROMBOSIS AND ATHEROSCLEROSIS?

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Summary Unlike arachidonic acid (eicosatetraenoic acid, C20:4ω-6, A.A.), eicosapentaenoic acid (C20:5ω-3, E.P.A.) does not induce platelet aggregation in human platelet-rich plasma (P.R.P.), probably because of the formation of thromboxane A_3 (T.X.A_3) which does not have platelet aggregating properties. Moreover, E.P.A., like A.A., can be utilised by the vessel wall to make an anti-aggregating substance, probably a Δ^{17}-prostacyclin (P.G.I_3). This finding suggests that, in vivo, high levels of E.P.A. and low levels of A.A. could lead to an antithrombotic state in which an active P.G.I_3 and a non-active T.X.A_3 are formed. Eskimos have high levels of E.P.A. and low levels of A.A. and they also have a low incidence of myocardial infarction and a tendency to bleed. It is possible that dietary enrichment with E.P.A. will protect against thrombosis.

Introduction

EPIDEMIOLOGICAL studies in North West Greenland over eight years have shown that the low incidence of acute myocardial infarction (A.M.I.) among Eskimos may be attributable to a delayed atherosclerotic process because their lipid and lipoprotein plasma fractions had both low levels of cholesterol and triglyceride and low levels of low density lipoprotein (L.D.L.) and very low density lipoprotein (V.L.D.L.) concentrations.[1,2] Furthermore, male Eskimos had high levels of high density lipoprotein (H.D.L.), which are associated with a low risk for A.M.I.[3,4] These plasma lipid and lipoprotein patterns were not genetic in origin but were clearly a result of the Eskimo diet.[5,6]

However, although delayed atherosclerosis may contribute to the low incidence of A.M.I. in Eskimos, other factors cannot be ruled out since Eskimos also have a bleeding tendency.[7,8]

The elucidation of how T.X.A_2 is formed in platelets and of its pro-aggregating role,[9] and the discovery of prostacyclin, a powerful anti-aggregating agent formed in the vessel wall,[10,11] has led to the suggestion that a balance between the formation of these two compounds controls platelet aggregability in vivo.[12] Both substances have the common precursor A.A.

A.A. is found in very small amounts in the plasma-lipids of Greenland Eskimos, whereas E.P.A. is present in fairly high concentrations[5] (see table). E.P.A. is transformed by platelet microsomes into T.X.A_3 which, unlike T.X.A_2, is not a pro-aggregating agent.[13]

Dyerberg and Bang[14] have suggested that the low incidence of A.M.I. in Eskimos could be partly due to a lack of the substrate required for the formation of pro-aggregating prostaglandins by the platelets. The present results show that E.P.A. is not pro-aggregatory in human platelets and that the vessel wall cyclo-oxygenase can use E.P.A. to synthesise an anti-aggregating agent, probably a prostacyclin of the "3" series (P.G.I_3).

Methods

Venous blood from human volunteers who had not taken aspirin for two weeks was collected in sodium citrate (0·11 mol/l), one part of citrate to nine parts of blood, and centrifuged at 160 *g* (5 min) to obtain platelet-rich plasma (P.R.P.).

In some experiments, washed human platelets, prepared as described before,[15] were used for aggregation studies. The formation of P.G.I_2-like activity by vascular rings was studied by the use of washed platelets from volunteers who had taken aspirin 1·5 g/day for three days before blood sampling. Inhibition of primary-phase aggregation to A.D.P. (2–5 μmol/l) or inhibition of aggregation induced by thrombin (0·04–0·4 U/ml) indicated prostacyclin-like activity.

Samples of thoracic and abdominal aorta were obtained

C20:5 AS PERCENTAGE OF TOTAL FATTY ACIDS IN LIPID FRACTIONS OF BLOOD IN ESKIMOS AND DANES[4]

	A.A. (C20:4)			E.P.A. (C20:5)		
	P.L.	C.E.	T.G.	P.L.	C.E.	T.G.
Eskimos	0·8	0·0	0·0	7·1	15·4	4·0
Danes	8·0	4·4	0·0	0·2	0·0	0·0

P.L.=phospholipid, C.E.=cholesterol esters, T.G.=triglycerides.

from freshly killed rats. Approximately 100 mg of arterial tissue was chopped and washed once in ice-cold "tris" buffer (0·05 mol/l, pH 7·5). After it had been shown to inhibit thrombin-induced platelet aggregation when added to the platelet cuvette, the tissue was washed several times in 10 ml of ice-cold tris buffer, then quickly frozen to −60°C, crushed to a coarse powder, and resuspended in five volumes of tris buffer. This suspension of vascular tissue was kept on ice during the experiment and used for incubation studies.

Platelet studies were carried out with A.D.P., the potassium salts of A.A. or E.P.A.,[16] or with thrombin in a coagulation apparatus 'Fibromate' (Bie & Berntsen, Copenhagen, Denmark) constructed with the cooperation of two of the authors (J. D. and E. S.). Aggregation in 300 µl of plasma at 37°C stirred magnetically at 800 r.p.m. in a cylindrical cuvette was recorded both turbidimetrically and nephelometrically. In other experiments a 'Payton' dual channel aggregometer (Payton Ass. Ltd., Ontario, Canada) was used with 500 µl P.R.P.

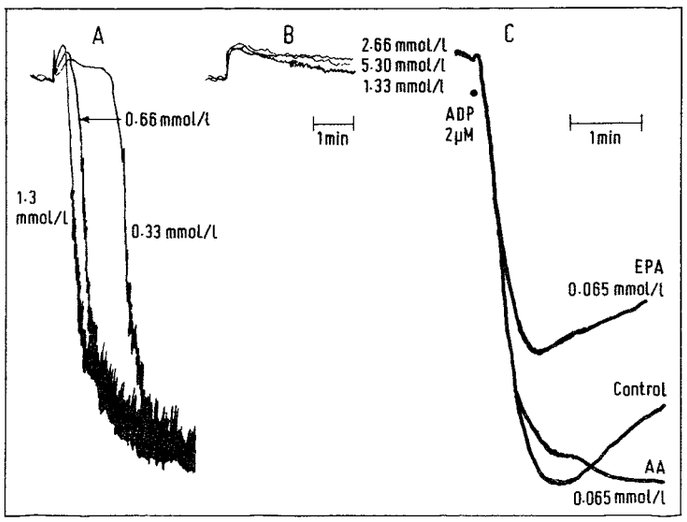

Fig. 1—Platelet aggregation induced in platelet-rich plasma by different concentrations of A.A. (A) and lack of pro-aggregation action of E.P.A. (B) at even higher concentrations.

Inhibitory effect of E.P.A. on the first phase of platelet aggregation induced by A.D.P. (2 µmol/l) in platelet-rich plasma. A.A. in the same concentrations has no inhibitory effect (C).

For the incubation studies with vascular tissue the potassium salts of A.A. and E.P.A. were prepared as described for the aggregation studies.

Results

Unlike A.A., E.P.A. did not induce aggregation in human P.R.P. in concentrations up to 5 times greater than that of A.A. (fig. 1). At lower concentrations (0·01–0·5 mmol/l), E.P.A. inhibited A.D.P.-induced aggregation to some extent. The anti-aggregating effect of E.P.A., however, was not due to its conversion to endoperoxides by cyclo-oxygenase for the effect was also seen with aspirin-treated platelets which were not aggregated by A.A. but were aggregated by thrombin and which showed the first-phase aggregation induced by A.D.P.

The presence of anti-aggregating activity in the suspension of vascular tissue was studied with platelets obtained from volunteers who had taken aspirin so that the platelets could not produce endoperoxides that could be utilised by the vascular tissue. Moreover, washed platelets were used to avoid any possibility of the vascular tissue utilising any A.A. in the plasma. Under these conditions anti-aggregating activity could be formed by the vascular tissue only from endogenous or exogenously added precursors. The initial suspension of vascular tissue (10–50 µl) inhibited thrombin-induced aggregation. This inhibitory activity was abolished when the tissue was repeatedly washed (5–20 times) and resuspended in fresh buffer (0·5 ml) (fig. 2). The inhibitory activity could then be restored by adding vascular tissue incubated with E.P.A. to the washed platelets from aspirin-treated volunteers (fig. 2). Since the restoration of anti-aggregating activity was prevented by pretreatment of the tissue with indomethacin (5–10 µg/ml), vessel wall cyclo-oxygenase was using E.P.A. to produce anti-aggregating activity.

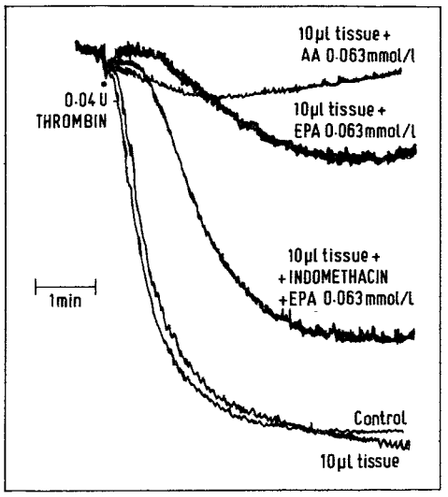

Fig. 2—Washed platelets from a volunteer who had taken aspirin, stimulated with thrombin (0·04 U/ml, control); thrombin plus suspension of vascular tissue washed several times (10 µl tissue suspension).

Inhibition of thrombin-induced aggregation occurred after incubating vascular tissue in platelet suspension either with E.P.A. (10 µl tissue suspension + E.P.A. 0·063 mmol/l) or A.A. (10 µl tissue suspension + A.A. 0·063 mmol/l). This inhibition was substantially blocked by pre-incubating the vascular tissue with indomethacin (10 µl tissue suspension + indomethacin + E.P.A. 0·063 mmol/l). Lack of complete aggregation was probably due to the direct anti-aggregation action of E.P.A. (see fig. 1).

The anti-aggregating activity formed could have been due to displacement of endogenous A.A. by E.P.A. and not to direct utilisation of E.P.A. However, when the same

Pro-aggregating	Platelets	Precursor	Vessel wall	Anti-aggregating
no	TXA_1	COOH (DHLA)	No PGI type compound	no
yes	TXA_2	Δ^5 COOH (AA)	PGI_2	yes
no	TXA_3	Δ^5 COOH (EPA)	PGI_3?	yes

Fig. 3—Structure of dihomo-γ-linolenic acid (D.H.L.A.), arachidonic acid (A.A.), and eicosapentaenoic acid (E.P.A.) from top to bottom, the biological products synthesised from them by platelets and vessel wall, and their biological properties.

concentrations of dihomo-γ-linolenic acid (C20:3) as that of E.P.A. were incubated with washed vascular tissue no anti-aggregating material was produced.

Discussion

A.A., the commonest precursor of prostaglandin synthesis, is transformed by the platelets into T.X.A_2, a potent pro-aggregating agent,[9] and by the vessel wall to prostacyclin (P.G.I_2).[10] The balance between the formation of these two compounds regulates platelet aggregability in vivo and hæmostatic plug formation.[12]

Our present results show that E.P.A. is not pro-aggregating in human P.R.P. even though T.X.A_3 is formed,[13] T.X.A_3 not being a pro-aggregating agent.[15] However, the vessel wall can utilise E.P.A. to synthesise a potent anti-aggregating agent, probably a Δ^{17}-prostacyclin (P.G.I_3).

Clearly, utilisation of E.P.A. rather than A.A. in vivo should displace the balance between pro-aggregating and anti-aggregating forces towards an anti-aggregating condition (fig. 3).

The fatty acid available for prostaglandin biosynthesis in the tissues of Greenland Eskimos (assuming a similar distribution as in plasma fatty acids) is mainly E.P.A. and not A.A., in contrast to that in Caucasians (table). The content of E.P.A. in the blood of Eskimos originates directly from the E.P.A. in the diet, since the dietary content of its possible precursor, linolenic acid (C18:3), is low.[6]

Microthrombus formation on areas of endothelial damage is thought to initiate events which lead to A.M.I.[17] The delayed atherosclerotic process in the vessel wall of Eskimos could be a consequence of several factors, including a favourable lipid profile and low aggregability of the platelets in vivo due to probably the presence of P.G.I_3 and to the lack of aggregating activity of T.X.A_3. Furthermore, the displacement of the balance of activity towards an anti-aggregating state could account for the hitherto unexplained observations of enhanced bleeding tendency in Eskimos.[7,8]

Dietary manipulation with dihomo-γ-linolenic acid, the precursor of prostaglandins of the "1" series has been suggested[18] as a means of preventing thrombosis since thromboxane A_1 (T.X.A_1) is not pro-aggregating and P.G.E_1 is anti-aggregating. However, this proposition was made before the discovery of prostacyclin and although still maintained by some,[19,20] it is now clear that the P.G.G_1 or P.G.H_1 are not substrates for the formation of the defensive substance, prostacyclin. E.P.A. has the Δ^5 double bond and can be converted into a potent anti-aggregating substance.

Dietary polyunsaturated fats are generally accepted as being "less harmful" than saturated ones. However, those used in for instance, margarines, are mainly oleic (C18:1) and linoleic acids (C18:2).[21] Linolenic acid (C18:3) is converted to E.P.A. in some species.[22] Thus, E.P.A. and possibly linolenic acid may be more appropriate as beneficial agents than "polyunsaturated fats" in general. Indeed, enrichment of tissue lipids with E.P.A., whether by dietary change or by supplementation may reduce the development of thrombosis and atherosclerosis in the Western World.

We thank Mrs E. A. Higgs for her help.

This study was supported by the Aalborg City Fund for medical research.

REFERENCES

1. Bang, H. O., Dyerberg, J., Nielsen, Aa., B. *Lancet*, 1971, i, 1143.
2. Bang, H. O., Dyerberg, J. *Acta med. scand.* 1972, **192**, 85.
3. Miller, G. L., Miller, N. E. *Lancet*, 1975, i, 16.
4. Miller, N. E., Førde, O. H., Thelle, D. S., Mjøs, O. D. *ibid*, 1977, i, 965.
5. Dyerberg, J., Bang, H. O., Hjørne, N. *Am. J. clin. Nutr.* 1975, **28**, 958.
6. Bang, H. O., Dyerberg, J., Hjørne, N. *Acta med. scand.* 1976, **200**, 69.
7. Berthelsen, A. *Meddr. Grønland*, 1940, **117**, 1.
8. Christensen, P. E., Schmidt, H., Jensen, O., Bang, H. O., Andersen, V., Jordal, B. *Acta med. scand.* 1953, **144**, 429.
9. Hamberg, M., Svensson, J., Samuelsson, B. *Proc. natn. Acad. Sci. U.S.A.* 1975, **72**, 2994.
10. Moncada, S., Gryglewski, R., Bunting, S., Vane, J. R. *Nature*, 1976, **263**, 663.
11. Johnson, R. A., Morton, D. R., Kinner, J. H., *et al. Prostaglandins*, 1976, **12**, 915.
12. Moncada, S., Vane, J. R. *Br. med. Bull.* 1978, **34**, 129.
13. Raz, A., Minkes, M. S., Needleman, P. *Biochim. biophys. Acta.* 1977, **488**, 305.
14. Dyerberg, J., Bang, H. O. *Lancet*, 1978, i, 152.
15. Vargaftig, B. B., Tranier, Y., Chignard, M. *Prostaglandins*, 1974, **8**, 133.
16. Schrör, K., Moncada, S., Ubatuba, F. B., Vane, J. R. *Eur. J. Pharmac.* 1978, **47**, 103.
17. Ross, R., Glomset, J. A. *New Engl. J. Med.* 1976, **295**, 421.
18. Willis, A. L., Comia, K., Kuhn, D. C., Paulsrud, J. *Prostaglandins*, 1974, **8**, 509.
19. Kernoff, P. B. A., Willis, A. L., Stone, K. J., Davies, J. A., McNicol, G. P. *Br. med. J.* 1977, ii, 1441.
20. *Ibid.* p. 1437.
21. Weibranch, J. L., Brignoli, C. A., Reeves, J. B., Iverson, J. L. *Food Technol.* 1977, *Feb.*, p. 80.
22. Crawford, M. A., Casperd, N. M., Sinclair, A. J. *Comp. Biochem. Physiol.* 1976, **54B**, 395.

β-ENDORPHIN IN HUMAN CEREBROSPINAL FLUID

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Summary β-endorphin is a brain peptide with potent morphine-like activity structurally related to the anterior pituitary hormone β-lipotrophin (β-L.P.H.). We have developed a radioimmunoassay for human β-endorphin in plasma and cerebrospinal fluid (C.S.F.). Since the antiserum also reacts with β-L.P.H., β-endorphin was distinguished by using a second antiserum which measures β-L.P.H. alone. With these two immunoassay systems and gel chromatography, we found β-endorphin in all 20 C.S.F. samples tested at a concentration always higher than, but with no other relationship to, that in plasma. β-endorphin was found in C.S.F. of patients who had hypopituitarism and undetectable plasma-β-endorphin, suggesting that it is synthesised in the brain rather than in the pituitary.

Introduction

β-ENDORPHIN may be the body's "endogenous opiate". This peptide is structurally related to β-lipotrophin (β-L.P.H.), a larger peptide first isolated in 1965,[1] which is found in the same cells of the anterior pituitary as