Res. clin. Stud. Headache, vol. 6, pp. 110–116 (Karger, Basel 1978)

Role of Individual Free Fatty Acids in Migraine

Michael Anthony

Division of Neurology, Prince Henry and Prince of Wales Hospitals, School of Medicine, University of New South Wales, Sydney

Introduction

Migraine is a low serotonin syndrome, in that an attack of headache is associated with serotonin release and plasma levels of the amine are significantly lower during headache than during headache freedom. As blood serotonin is found mainly in the platelets, lower plasma levels reflect serotonin release from the platelet cells. There is evidence to suggest that the release reaction during migraine is mediated through a serotonin releasing factor, which appears in the blood during the attack (*Anthony et al.*, 1969). This factor has a molecular weight of less than 50,000, which is against its being a protein, an antigen-antibody complex or some other form of large molecule. On the other hand, free fatty acids (FFA), various amino acids, polypeptides, prostaglandins or monoamines, e.g. tryptamine, tyramine and catecholamines, qualify as candidates for platelet serotonin releasers in migraine (*Anthony and Lance*, 1975).

Certain FFA, particularly stearic, palmitic, linoleic and behenic, are known to be quite potent in releasing platelet serotonin *in vitro* (*Inouye et al.*, 1970). Several patients relate their migraine attacks to the ingestion of fatty meals and two studies, so far, have demonstrated a significant rise in total plasma FFA during attacks of migraine (*Hockaday et al.*, 1971; *Anthony*, 1976).

The purpose of this study is to identify which of the four most commonly occurring plasma FFA – stearic, palmitic, oleic and linoleic – rises most during migraine and which could conceivably be responsible for the significant serotonin release which occurs *in vivo*, during the process of migraine headache. Table I shows a list of FFA in normal subjects, with the highest concentration in plasma.

Table I. Normal subjects; plasma levels free fatty acids

	%	mg/l	nmol/ml
Total	–	290	1,035.3
Palmitic	24.9	80.9	288.8
Stearic	14.9	43.2	154.2
Oleic	25.5	73.9	264.0
Linoleic	13.1	38.0	135.6
Palmitoleic	7.2	20.9	74.5
Arachidonic	2.4	6.9	24.8

Materials and Methods

10 patients, with frequent and severe migraine headaches, were admitted to the hospital for study. All interval medication was stopped for 1 week and no ergot preparations or alkaloid analgesics were permitted for 3 days prior to admission.

Blood was collected three times daily, before and after the migraine attack, and at 4-hour intervals during the headache. Patients were allowed the standard ward diet, but were not permitted to have food or sweet fluids between meals. Blood was collected a few minutes before the three main meals, after the patient had rested for about 20 min. The purpose of the above procedures was to offset the possible effects of diet, posture and exercise on plasma FFA.

Blood was collected into a chilled tube, containing 2% EDTA as an anti-coagulant. It was spun at low speed to separate the cellular elements and then the supernatant platelet-rich plasma was separated and spun at 2,000 rpm at 4 °C for 15 min to isolate the platelets. The platelet-poor plasma was used for FFA estimation, whilst the platelet button was used to estimate platelet serotonin.

Total plasma FFA levels were estimated by a modification of Dole's extraction method (*Trout et al.*, 1960). Individual FFA were estimated by gas liquid chromatography (GLC), using ethylene glycol succinate as the liquid phase into which were injected 2 μl of the methylated plasma extract, using methyl-8. The oven temperature of the GLC instrument (Packard – model 417) was set at 180 °C, injection ports at 240 °C and the detectors were maintained at 210 °C. Platelet serotonin was estimated by a fluorometric technique (*Crawford and Rudd*, 1962).

Results

Total Plasma Free Fatty Acids

Higher levels of more than 10% of FFA were found in 8 patients during the headache period. The highest rise, when pre-headache and headache values were compared, was 118%, the mean rise for the group being 42.6%. Mean values for pre-headache, headache and post-headache periods were 340, 485 and 322 nmol/ml, respectively. Statistical comparison between headache, pre- and

Table II. Migraine; total plasma free fatty acids

	Pre-headache	Headache	Post-headache
Mean levels, nmol/ml	340	485	322
Observations	34	60	34
Statistical significance	$p < 0.001$		$p < 0.001$
Number of patients		10	
Patients showing rise		8	
Mean rise pre-headache/headache, %		42.6	

Table III. Migraine; individual plasma free fatty acids; 10 patients (mean values, nmol/ml)

	Stearic	Palmitic	Oleic	Linoleic
Pre-headache	115.9	83.2	143.0	41.3
Headache	157.6	150.0	278.8	98.1
Post-headache	111.2	91.4	156.3	72.6
Mean rise pre-headache/headache, %	36.0	80.3	95.0	137.5

post-headache periods showed a highly significant difference ($p < 0.001$; table II).

Individual Plasma Free Fatty Acids

All patients showed a rise in all FFA during headache, except one, who failed to demonstrate any change in levels of stearic and palmitic acids. When pre-headache and headache values were compared, the order of magnitude of rise of individual FFA was stearic (36.0%), palmitic (80.3%), oleic (95.0%) and linoleic (137.5%). The results are summarized in table III and the difference between the pre-headache, headache and post-headache periods was highly significant.

Platelet Serotonin

Of the 10 patients, 8 demonstrated a significant fall in platelet serotonin content during the headache period. The mean fall for the group, when pre-headache and headache values were compared, was 22%, whilst the mean values for the pre-headache, headache and post-headache periods were 462, 361 and 441 ng/10^9 platelets. A statistical comparison of the three periods showed the

Table IV. Migraine; platelet serotonin

	Pre-headache	Headache	Post-headache
Mean levels, ng/10^9 platelets	462	361	441
Number of observations	35	63	41
Statistical significance	$p < 0.001$		$p < 0.001$
Number of patients		10	
Patients showing fall		8	
Mean fall pre-headache/headache, %		22.0	

lower values during the headache period to be highly significant. Details of the results are shown in table IV.

Discussion

The results of this investigation show that in the majority of patients a migraine attack is accompanied by a rise in total plasma FFA and a simultaneous fall of platelet serotonin. It further demonstrates that of the commonly occurring FFA in plasma, the two saturated acids, stearic and palmitic, and the unsaturated oleic acid, show a less significant rise during the attack than the unsaturated linoleic acid, the latter showing a rise of 137.5%. This last observation assumes significance when it is remembered that linoleic acid is the precursor of all prostaglandins. It can be converted to arachidonic acid from which prostaglandin E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$) are derived or to dihomo-linoleic acid, which gives rise to PGE_1 and $PGF_{1\alpha}$ (*Weeks,* 1969).

Of the naturally occurring prostaglandins, PGE_1 is the most powerful vasodilator. It depresses smooth muscle contraction in resistance vessels during intravenous infusion, thus causing headache in man (*Carlson et al.,* 1968). Dilatation of the cranial circulation in the dog (*Denton et al.,* 1972) and differential dilatation in the cranial circulation of the monkey, affecting the external more than the internal circulation, has also been demonstrated following intracarotid infusion of PGE_1 (*Spira et al.,* 1976).

If raised levels of linoleic acid in plasma during migraine contribute towards increased prostaglandin formation, it is quite possible that they are also capable of causing the release of platelet serotonin. Alternatively, the release reaction could be affected through the activity of prostaglandin endoperoxides, which are intermediary compounds in the biosynthesis of prostaglandins, with a half-life of about 5 min (*Samuelson,* 1976). Serotonin is known to be vasotonic to the circulation (arteries and resistance arterioles) and reduction of its circulating

levels would only tend to reduce vascular tone which, coupled with the vasodilating effects of the E prostaglandins, can only lead to pathological vasodilatation in the carotid system, which manifests itself clinically as headache.

It would not be unreasonable, at this stage, to speculate on the mechanism of action of two common trigger factors in migraine – emotional upsets and ingestion of alcohol; both these factors release catecholamines which, in turn, through stimulation of β-adrenoreceptors release FFA with their consequent effects on prostaglandin synthesis and platelet serotonin stores. It would, therefore, be most interesting to estimate plasma FFA, catecholamines and PGE_1 levels during spontaneous migraine, as well as following emotional stress or ingestion of alcohol, to see their interrelationship. Information already exists as to increases in levels of plasma FFA (*Hockaday et al.*, 1971; *Anthony*, 1976) and catecholamines (*Hsu et al.*, 1978) during spontaneous episodes of migraine. This can be extended to encompass induced migraine. As far as PGE_1 is concerned, no reliable information is available, as estimation of natural PGE_1, with a half-life of less than 30 sec (*Cornette et al.*, 1974) cannot be used to assess endogenous rates of formation, since the substance is also formed during the process of blood collection and preparation of plasma by platelet activating systems, as well as by non-enzymatic cyclization of unsaturated FFA in blood. On the other hand, prostaglandin metabolites (e.g. 15-keto-dihydro derivatives) are more stable, with a half-life of about 30 min, cannot be synthesized in the blood and, therefore, more accurately reflect endogenous rates of prostaglandin formation (*Samuelson and Green*, 1974).

To complement the observations of the above study it appears necessary to (a) assess accurately the serotonin releasing ability on isolated platelets of the four FFA investigated so far, and (b) administer the above four FFA separately to migrainous patients, to see whether they are capable of inducing migraine attacks, whilst at the same time assessing their effect on the serotonin content of the patient's platelets. Such a project is now in progress.

Summary

Total plasma free fatty acids, platelet serotonin content and plasma stearic, palmitic, oleic and linoleic acids were estimated in 10 migraine patients before, during and after a migraine attack. Total and individual plasma free fatty acid levels rose and platelet serotonin content fell in most patients. The highest rise was observed in linoleic acid, which is known to be a potent liberator of platelet serotonin *in vitro* and is the only precursor of all prostaglandins in the body. It is suggested that the rise in plasma levels of linoleic acid in migraine could be responsible for the platelet serotonin release observed during the attack. At the same time, it may also serve as a source of increased prostaglandin E_1 synthesis, which has a powerful vasodilating effect.

It is realized that both suggestions have to be confirmed by relevant investigations, as outlined in the body of this paper.

References

Anthony, M.: Plasma free fatty acids and prostaglandin E_1 in migraine and stress. Headache *16:* 58–63 (1976).

Anthony, M.; Hinterberger, H., and Lance, J.W.: The possible relationship of serotonin to the migraine syndrome. Res. clin. Stud. Headache, vol. 2, pp. 29–59 (Karger, Basel 1969).

Anthony, M. and Lance, J.W.: The role of serotonin in migraine; in Pearce, Modern topics in migraine, pp. 107–123 (Heinemann, London 1975).

Carlson, L.A.; Ekelund, L.G., and Oro, L.: Clinical and metabolic effects of different doses of prostaglandin E_1 in man. Acta med. scand. *183:* 423–430 (1968).

Cornette, J.C.; Harrison, K.L., and Kirton, K.T.: Measurement of prostaglandin $F_{2\alpha}$ metabolites by radioimmunoassay. Prostaglandins *5:* 155–165 (1974).

Crawford, N. and Rudd, B.T.: A spectrofluorometric method for the determination of serotonin (5-hydroxytryptamine) in plasma. Clinica chim. Acta *7:* 114–121 (1962).

Denton, I.C., jr.; White, R.P., and Robertson, J.T.: The effects of prostaglandins E_1, A_1 and $F_{2\alpha}$ on the cerebral circulation of dogs and monkeys. J. Neurosurg. *36:* 34–42 (1972).

Hockaday, J.M.; Williamson, D.H., and Whitty, C.W.M.: Blood glucose levels and fatty acid metabolism in migraine related to fasting. Lancet *i:* 1153–1156 (1971).

Hsu, L.K.G.; Crisp, A.H.; Kalucy, R.S.; Koval, J.; Chen, C.N.; Carruthers, M., and Zilkha, K.J.: Nocturnal plasma levels of catecholamines, tryptophan, glucose and free fatty acids and the sleeping encephalographs on subjects experiencing early morning migraine: in Greeve, Current concepts on migraine research, pp. 121–130 (Raven Press, New York 1978).

Inouye, A.; Shio, H.; Sorimachi, M., and Katoaka, K.: Unsaturated fatty acids: platelet-serotonin releasers in tissue extracts. Experientia *26:* 308–309 (1970).

Samuelson, B.: PG endoperoxides and thromboxanes; in Prostaglandins and therapeutics, vol. 2, No. 3, pp. 1, 4 (Upjohn, Kalamazoo 1976).

Samuelson, B. and Green, K.: Endogenous levels of 15-keto-dihydro-prostaglandins in human plasma. Parameters for monitoring prostaglandin synthesis. Biochem. Med. *11:* 298–303 (1974).

Spira, P.J.; Mylecharane, E.J., and Lance, J.W.: The effects of humoral agents and anti-migraine drugs on the cranial circulation of the monkey. Res. clin. Stud. Headache, vol. 4, pp. 37–75 (Karger, Basel 1976).

Trout, D.N.; Estes, E.A., and Friedberg, S.J.: Titration of free fatty acids of plasma: a study of current methods and a new modification. J. Lipid Res. *1:* 199–202 (1960).

Weeks, J.R.: The prostaglandins: biologically active lipids with implications in circulatory physiology. Circulation Res. *24–25:* suppl. 1, pp. 123–129 (1969).

Dr. M. Anthony, Division of Neurology, School of Medicine, University of New South Wales, The Prince Henry Hospital, Little Bay, Sydney, NSW 2036 (Australia)

Discussion

Gawel: Adrenaline and noradrenaline have been shown by *Hsu* and his colleagues to be released prior to early morning migraine. In our experiments with adrenaline and noradrenaline infusions we found a rise in FFA, which would fit in with your findings

during migraine attacks. Could you discuss this effect? Could adrenaline or noradrenaline be the platelet damaging substance? In some patients we measured catecholamines and they were not elevated. Adrenaline is a weak releaser of 5-HT.

Anthony: We measured plasma catecholamine levels in the first 10 patients but found no significant difference in the pre-headache, headache and post-headache periods. Catecholamines are relatively weak releasers of platelet serotonin compared to FFA as far as I know.

Appenzeller: What is the situation regarding effort migraine and FFA release?

Anthony: FFA are not significantly higher in effort migraine.

Korczyn: In his elegant paper, Dr. *Anthony* has demonstrated that FFA may be responsible for serotonin release. However, some patients' platelets were resistant. This raises the question as to whether the defect in migraine is in the plasma or in the platelets. Incidentally, we have repeated *Anthony*'s earlier observations and I can confirm that a plasma factor exists during migraine attacks which can release serotonin from platelets. Moreover, we have shown that this factor is active also on homologous platelets. So, the abnormality in migraine responsible for serotonin release seems to reside in the plasma rather than in the platelets.