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Alteration of Δ-6 desaturase by vitamin E in rat brain and liver

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Δ-6 Desaturase, measured at substrate saturation using linoleic acid, was found to be increased by more than two-fold when the content of vitamin E in brain microsomal membrane suspension was increased (up to 7.5 μg/mg membrane protein, i.e. 100 μg/g tissue from which microsomes were prepared). In contrast, this activity was reduced by 25% in the liver. This raises the question of the multiple role of vitamin E in membranes, the control of membrane polyunsaturated fatty acids through synthesis, and their protection against peroxidation.

Vitamin E is the only efficient lipid-soluble chain-breaking antioxidant in biological membranes [8, 9]. Indeed, its principal function is to scavenge free radicals in the lipid phase, protecting the polyunsaturated fatty acids against peroxidation, induced mainly by free radicals. In addition, other specific effects of vitamin E on membrane architecture by physico-chemical interaction with specific polyunsaturated fatty acids or by control of their lipid profile have been suggested [8, 10, 13]. However, the direct effect of vitamin E on polyunsaturated fatty acid biosynthesis has not been examined, although it is known that Δ-6 desaturase enzymatic activity is altered by various factors [22].

As nervous tissue contains very high amounts of structural lipids in the membranes and is rich in vitamin E [34, 35], it is assumed that this vitamin plays a very important role in the nervous system [25, 30]. Indeed, vitamin E is very important for the maintenance of normal neurological function in man as well as in experimental animals [30–32]. In fact, dietary levels of vitamin E can control vitamin E concentration in various tissues, including brain [5, 11, 15, 24].

Female Sprague–Dawley rats were fed standard chow containing 1900 mg 18:2 (n-6) and 130 mg 18:3 n-3 per 100 g (Iffa-Credo, France). Its lipid, fatty acid, and vitamin E content have been previously described [5, 11]. Pups were sacrificed at 6 days by decapitation. Brain and liver were dissected out, weighed, cut into small pieces and homogenized in a Potter in buffer containing saccharose (0.25 M), Na_2HPO_4 (0.05 M), glutathion (2 mM) pH 7.4 (5 ml per g of tissue). Cell debris, nuclei and mitochondria were discarded after centrifugation at 12,000×*g*. Protein in the supernatant was measured according to Lowry [23].

Δ-6 Desaturase was determined as described [3] in the 12,000×*g* supernatant from brain and liver homogenate (crude microsomal suspension). A suspension was used and not a crude microsomal pellet as it is known that microsomes are poorly active unless cytosol is added. The cytosolic factor structure and function is not known in any organ: thus it is not possible to use microsomal membranes with added cytosol (reconstituted fraction). The membrane-bound desaturase acts in conjunction with cytochrome *b5* and cytochrome *b5* reductase and up to now attempts at purification have been unsuccessful; thus it was not possible to use the purified enzyme.

Incubations were performed as previously described [3]. Briefly, tissues were homogenized in 0.25 M saccharose, 0.05 M phosphate and 2 mM glutathione, pH 7.4 (2 g fresh weight tissue/5 ml buffer). Homogenates were centrifuged for 15 min at 12,000×*g*, and supernatants were carefully taken up. Incubation media (2 ml) contained variable amounts of protein, and (in mM) Na_2HPO_4 50, APT 7.5, $MgCl_2$ 3.8, NADPH 0.2, NADH 0.5, CoA 0.2, and [1-^{14}C] linoleic acid (100 nmol, 2μCi, 20 μl). After 30 min, incubations were stopped by addition of 2 N KOH in ethanol. Unlabelled fatty acids (commercial grade) were added as carriers and lipids were saponified. After acidification, fatty acids were extracted twice with 5 ml of hexane and were methylated with 14%

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boron trifluoride in methanol for one hour. Methyl esters were purified by thin-layer chromatography using petroleum hydrocarbon (b.p. 40–60°C)/diethyl ether (80:20, v/v) as developing solvent. Radioactive methyl esters were visualized by autoradiography, the fractions were extracted twice with 3 ml of hexane and twice with diethyl ether, and the lipids were further resolved according to their degree of unsaturation by argentation thin-layer chromatography [3] (30% $AgNO_3$). After development with petroleum hydrocarbon (b.p. 40–60°C)/diethyl ether (50:50, v/v), radioactivity in fatty acids was determined using automatic scanning (Berthold).

For vitamin E incorporation, the 12,000×*g* supernatant (1 ml for liver and 2 ml for brain) was incubated for 15 min at 4°C with 0.01 ml of different concentrations of vitamin E in ethanol (vitamin E, DL-α-tocopherol, was obtained from Sigma). The suspension was then centrifuged for 1 h at 100,000×*g*. Vitamin E was determined in the pellet according to Katsui's method [18] with slight modifications as previously published [12]. For HPLC determination, a 12.5-cm-long lichosphere RP 18 column containing 4 μm particles was used (Merck Clevenot, France). The eluant, methanol-water (95:5), was pumped at a rate of 1 ml/min with a 2150 LKB pump. Vitamin E was detected by fluorescence (excitation 295 nm, emission 320 nm) using a Schoffel FS 970 fluorometer to eliminate the UV-absorbing material migrating close to vitamin E, and concentration calculated with an integrator (Delsi, France).

All the data previously determined in mouse [3] were measured for rat in this study. Linearity with protein concentration, and linoleic acid concentration, and length of incubation were found to be similar with both animals.

The relationship between vitamin E added in the test tube and its concentration in the centrifuged suspension of membranes (crude microsomes) was found to be linear under the conditions used ($r = 0.9860$) : 37% of the added vitamin E being incorporated within 15 min. In liver, an overnight incubation resulted in 100% incorporation [21], but was not compatible with maintaining measurable desaturating activity.

Fig. 1 shows that brain Δ-6 desaturase increased by more than two-fold when vitamin E content was increased up to 7.5 μg/mg protein; then activity slightly decreased up to 15 μg/mg protein and remained constant at high vitamin E content. In contrast, increasing amount of vitamin E in liver decreased delta-6 desaturase activity. In Fig. 1, the *x* axis origin corresponds to no addition of vitamin E in the incubated suspensions. However they contain physiological amounts of vitamin E: 25 μg/g fresh weight of liver according to Bucher and

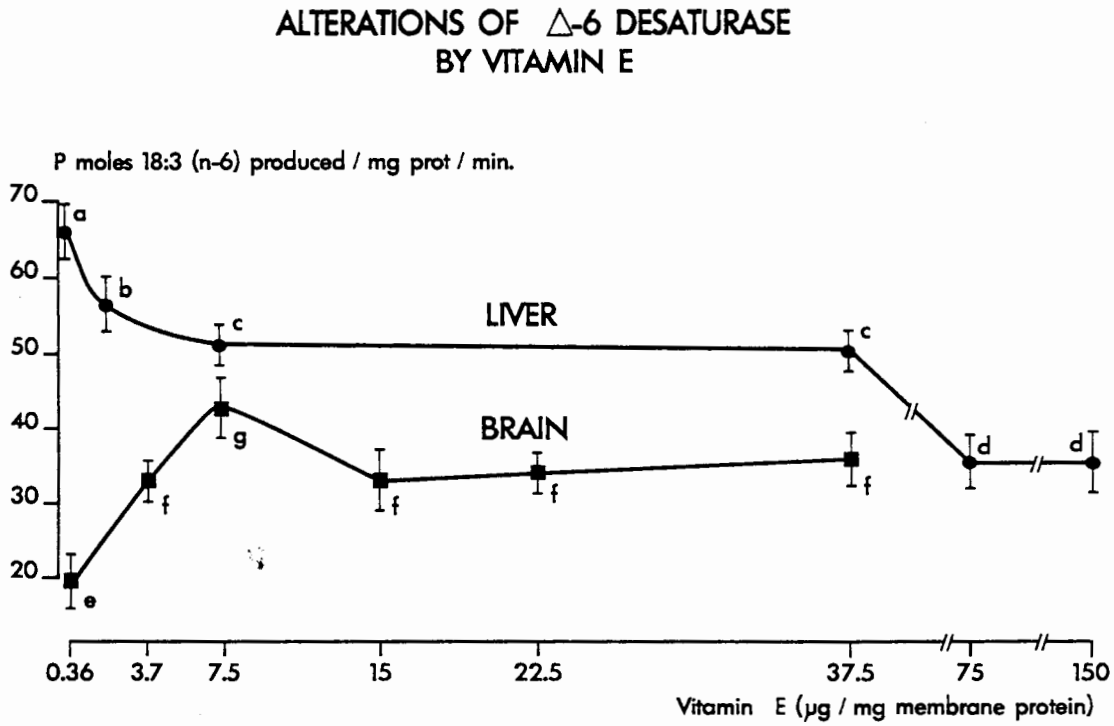

Fig. 1. Alterations of linoleic acid Δ-6 desaturase in brain and liver by vitamin E. Each point is the mean value of at least 3 independent measurements. Bars are standard errors of at least 3 measurements. Statistical analysis was performed using ANOVA. Values not bearing the same superscript letter are significantly different at $P < 0.05$.

Roberts [7] and confirmed in this study: 0.35 μg/mg centrifuged protein suspension, i.e. a crude microsomal preparation; 5 μg/g fresh weight of brain (0.38 μg/mg crude microsomal proteins), according to our previous results [12]. Thus, 100 μg/g vitamin E added in the test tube containing the tissue corresponded to 7.5 μg/g crude microsomal proteins.

Δ-6 Desaturase was not affected by 1% ethanol in the test tube (increasing amounts of vitamin E were dissolved in a constant volume of ethanol, so as to add the same volume of ethanol in all experiments).

It has been found that Δ-6 desaturase is altered by numerous factors such as cholesterol, ethanol, trans-isomer fatty acids, n-3 fatty acids, and various hormones and some pathophysiological conditions such as diabetes or obesity [22]. However, the effect of vitamin E has not been evaluated so far. Thus this work demonstrates for the first time that vitamin E affects desaturation of polyunsaturated fatty acids in the brain and the liver. Interestingly, the alterations are inverse.

Vitamin E is more easily incorporated in membranes when animals are fed a vitamin E-deficient diet [28]. Moreover, added vitamin E decreases membrane fluidity in liposomes [33], in rat erythrocytes [17] and in rat lung microsomes [28]. In addition, Δ-6 desaturase seems to be sensitive to membrane fluidity, and a negative correlation has been found between membrane fluidity and Δ-6 desaturation [6]. Thus it is more easy to explain increased Δ-6 desaturase in brain than the observed decrease in liver: incorporation of the vitamin in the membrane decreases fluidity and thus increases Δ-6 desaturase, protects the enzyme against peroxidation and increases activity by acting as an electron donor as suggested by Infante [16]. Moreover, vitamin E has been found to alter other enzymatic activities: in vitro, it inhibits platelet phospholipase [14], reduces pig brain adenosine triphosphatase [19] and inhibits potato tuber 5-lipoxygenase [29]. Interestingly, when in excess or deficient, vitamin E reduces Δ-9 desaturation [27].

It cannot be excluded that vitamin E, as a lipid compound, may affect the activity of membrane bound enzymes by unspecific mechanisms under the conditions used. However, it is noteworthy that the effects are opposite in brain and liver.

Moreover, how specific these observations are for vitamin E is still unclear: it would be interesting to test the effect of other vitamins, or substances structurally related to vitamin E, or non-structurally related antioxidants.

It has been shown (not determined in brain) that dietary vitamin E deficiency alters the quantity of polyunsaturated fatty acids in various tissues, with an increase in arachidonic acid [9, 20, 26]. The decrease in some polyunsaturated fatty acids has been explained by increased peroxidation, but increased desaturating activity has been proposed to explain increased quantities.

It has been suggested that vitamin E not only acts to protect polyunsaturated fatty acid against peroxidation [13] but also plays a role in the membrane architecture. The relationship between vitamin E and polyunsaturated fatty acids is not clear. In this respect we have shown that peripheral nerve contains a much larger quantity of vitamin E than that needed for protecting membrane polyunsaturated fatty acids [12]; there is no correlation between alteration of vitamin E content and myelination or polyunsaturated fatty acid content in the neurological dysmyelinating trembler mutant [4]. Finally, in sciatic nerve during development and aging, there is a relationship between vitamin E content and (n-6) polyunsaturated fatty acids, but not between vitamin E and (n-3) fatty acids [12].

Thus, this work suggests that vitamin E plays additional roles in relation to membrane polyunsaturated fatty acids and raises the question of the side effects of pharmacological doses of vitamin E.

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