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Increased arachidonic acid concentration in the brain of Flinders Sensitive Line rats, an animal model of depression

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Abstract Depression may be associated with impaired membrane PUFA composition, especially decreased n-3 PUFA. This assumption has not been tested at the level of brain tissue. Moreover, most studies were confounded by dietary variability. We examined the FA composition of selected brain areas in an animal model of depression, the Flinders Sensitive Line (FSL) rat, and compared the findings with those in controls fed identical diets. In all brain regions studied, the concentration of arachidonic acid (AA) was significantly higher in the FSL rats: in the hypothalamus by 21%, in the nucleus accumbens by 24%, in the prefrontal cortex by 31%, and in the striatum by 23%. No significant differences were observed for n-3 PUFA or for the saturated and monounsaturated FAs. Our results confirm the existence of altered brain PUFA composition in an animal model of depression. The finding of increased AA, an n-6 PUFA, rather than decreased n-3 PUFA, emphasizes the importance of both PUFA families in the pathophysiological processes underlying depression. The FSL rat is a useful tool for further elucidation of the FA disturbances in depression.—Green, P., I. Gispan-Herman, and G. Yadid. **Increased arachidonic acid concentration in the brain of Flinders Sensitive Line rats, an animal model of depression.** ***J. Lipid Res.*** **2005.** 46: **1093–1096.**

Supplementary key words n-6 polyunsaturated fatty acids • n-3 polyunsaturated fatty acids • depression • docosahexaenoic acid • eicosapentaenoic acid

Depression may be associated with a dietary deficiency of n-3 PUFA. This assumption was strengthened by the results of epidemiological studies (1) as well as correlational studies of erythrocyte phospholipid fatty acid composition, giving rise to the "phospholipid hypothesis" of depression (2). Researchers reported that n-3 PUFA concentration in the red blood cell membrane of depressive patients was lower than in controls (3, 4) and that it correlated negatively with the severity of depression (5). Others demonstrated that the ratio of arachidonic acid (AA), an n-6 PUFA, to eicosapentaenoic acid (EPA), an n-3 PUFA, in erythrocytes and plasma of depressive subjects correlated positively with the severity of depression (5). Trials of n-3 PUFA supplementation to depressive patients as a means of achieving relatively harmless psychotropic effects were reported as well (6, 7).

Although very attractive, the phospholipid hypothesis has not been tested at the level of the brain in depressive human subjects. Moreover, most of the clinical studies to date were confounded by dietary variability. The availability of an animal model of depression offers the opportunity to overcome both of these problems. The Flinders Sensitive Line (FSL) was established by genetically selecting (breeding) Sprague-Dawley rats for supersensitive hypothermic responses to cholinergic agents (8, 9). FSL rats show reduced appetite and general activity in addition to an increased amount of and reduced latency to rapid eye movement sleep, all behaviors that resemble the characteristic symptoms of patients diagnosed with major depression. Both the behavioral and neurochemical alterations in FSL rats were found to be normalized after chronic treatment with antidepressants (8, 9).

In the present study, we used this unique animal model of depression to test the phospholipid hypothesis at the level of the depressive brain. Specifically, the fatty acid composition of selected brain regions from FSL rats was compared with that of normal control Sprague-Dawley rats fed an identical diet.

Manuscript received 20 January 2005 and in revised form 14 March 2005.

Published, JLR Papers in Press, April 1, 2005.
DOI 10.1194/jlr.C500003-JLR200

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl ester; FSL, Flinders Sensitive Line.

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TABLE 1. Composition of the major fatty acid classes

	Hypothalamus		Nucleus Accumbens		Prefrontal Cortex		Striatum	
Fatty Acid Group	Sprague-Dawley	FSL	Sprague-Dawley	FSL	Sprague-Dawley	FSL	Sprague-Dawley	FSL
Saturated	43.30 ± 1.57	43.61 ± 1.67	45.83 ± 1.12	45.46 ± 3.38	47.25 ± 1.67	46.21 ± 2.06	43.60 ± 1.49	42.30 ± 2.71
MUFA[a]	26.93 ± 1.82	26.03 ± 1.07	21.51 ± 1.23	20.75 ± 1.97	18.52 ± 2.12	17.33 ± 1.48	25.65 ± 2.39	25.44 ± 3.82
n-6 PUFA[b]	15.55 ± 0.73	17.06 ± 0.5[c]	17.73 ± 0.63	19.69 ± 1.04[c]	17.77 ± 0.57	20.53 ± 1.40[c]	15.21 ± 0.58	16.84 ± 0.51[d]
n-3 PUFA[e]	14.05 ± 0.90	12.44 ± 0.88	14.66 ± 0.23	13.57 ± 1.11	15.75 ± 0.33	14.99 ± 0.86	15.15 ± 1.22	14.69 ± 0.72

FSL, Flinders Sensitive Line. Values are means ± SD of the fatty acid sums, expressed as weight percentages of total fatty acids (n = 5).
[a] MUFAs include 16:1, 18:1, 20:1, 22:1, and 24:1.
[b] n-6 PUFAs include 18:2, 20:3, 20:4, 22:4, and 22:5.
[c] $P < 0.01$.
[d] $P < 0.005$.
[e] n-3 PUFAs include 20:5, 22:5, and 22:6.

METHODS

FSL and Sprague-Dawley (control) rats (230–250 g; Bar-Ilan University) served as subjects in these experiments. The animals were maintained on a 12 h light/12 h dark cycle (lights off at 7:00 AM) with food and water available ad libitum. All experimental procedures were approved by the University Animal Care and Use Committees and were done in accordance with National Institutes of Health guidelines.

Punches were taken from four brain regions (hypothalamus, nucleus accumbens, prefrontal cortex, and striatum) of anesthetized rats as described previously (10). The brain tissue was immediately frozen in liquid nitrogen, weighed, and kept at −80°C. Lipids were extracted from brain tissue by homogenization in cold hexane-2-propanol (3:2, v/v) containing 5 mg/100 ml butylated hydroxytoluene as an antioxidant and 5 mg/100 ml heneicosanoic acid (21:0) as an internal standard, essentially as described previously (11). Transmethylation of fatty acids and gas chromatographic analysis of the fatty acid methyl esters (FAMEs) were performed as described previously (12). In brief, fatty acids were converted to FAMEs by heating with BF_3 in methanol; the methyl esters were separated on capillary columns in an HP 5890 Series II gas chromatograph equipped with a flame ionization detector. Peak areas were integrated and plotted with the aid of the Varian Star Integrator computer package. Identification of individual FAMEs was made by comparing retention times with those of authentic standards.

Statistical analysis

Individual fatty acids are expressed as weight percentages of total identified fatty acids (means ± SD). Differences between groups were analyzed by the Mann-Whitney test.

RESULTS

Analysis of the fatty acid classes in the study and control rats demonstrated a significantly higher concentration of n-6 PUFA in the brains of the FSL rats (**Table 1**). This difference was attributable mainly to the AA concentrations, which were greatly increased in the FSL rats compared with the Sprague-Dawley controls in all brain regions examined (**Fig. 1**): in the hypothalamus by 21%, in the nucleus accumbens by 24%, in the prefrontal cortex by 31%, and in the striatum by 23%. The concentrations of the other two long-chain n-6 PUFAs, 22:4n-6 and 22:5n-6, were also significantly different between the groups, but their levels were lower in the FSL rats than in the controls (**Table 2**). No differences were observed for n-3 PUFA, although mean docosahexaenoic acid (DHA) levels were slightly and nonsignificantly lower in all brain regions of the FSL rats (hypothalamus, 11.05 ± 0.71 and 12.43 ±

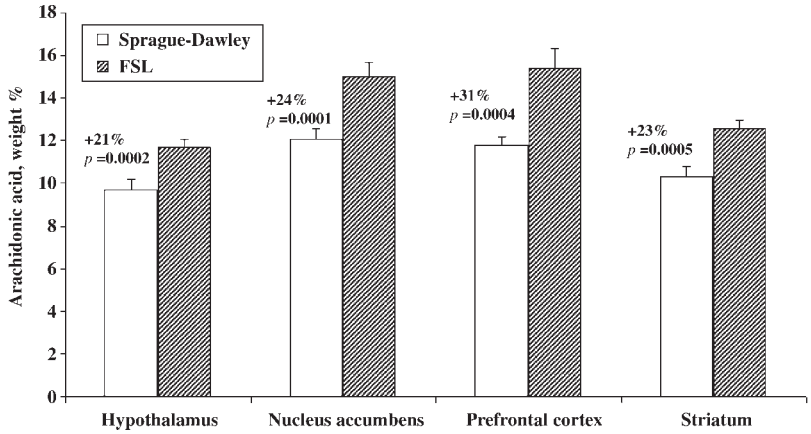

Fig. 1. Arachidonic acid (AA) concentration of selected brain areas. Values from control (open bars) and Flinders Sensitive Line (FSL; hatched bars) rats are expressed as weight percentages (means + SD; n = 5). The percentage increase of AA in the FSL rats and the *P* value for each comparison are shown above the bar pairs.

TABLE 2. n-6 PUFA composition of the brain regions

	Hypothalamus		Nucleus Accumbens		Prefrontal Cortex		Striatum	
Fatty Acid	Sprague-Dawley	FSL	Sprague-Dawley	FSL	Sprague-Dawley	FSL	Sprague-Dawley	FSL
22:4 n-6	4.10 ± 0.11	3.86 ± 0.20	3.57 ± 0.12	3.15 ± 0.39	3.71 ± 0.22	3.36 ± 0.41	3.01 ± 0.11	2.72 ± 0.15[a]
22:5 n-6	0.56 ± 0.10	0.33 ± 0.02[b]	0.70 ± 0.14	0.38 ± 0.04[b]	0.88 ± 0.12	0.54 ± 0.04[b]	0.45 ± 0.04	0.33 ± 0.10

Fatty acids (means ± SD) are expressed as weight percentages of total fatty acids (n = 5).
[a] $P < 0.05$.
[b] $P < 0.005$.

1.11 weight%, respectively; nucleus accumbens, 12.39 ± 1.21 and 13.48 ± 0.44 weight%, respectively; prefrontal cortex, 14.66 ± 0.80 and 15.31 ± 0.29 weight%, respectively; and striatum, 12.67 ± 1.01 and 13.09 ± 1.44 weight%, respectively). No differences were observed in EPA levels (data not shown). Similarly, no differences were observed for the saturated and monounsaturated fatty acids (Table 1).

To estimate the final biosynthetic pathway of the n-6 PUFA and n-3 PUFA families, we calculated the ratio of 22:5n-6 to 22:4n-6 and the ratio of 22:6n-3 to 22:5n-3 (**Table 3**). These ratios reflect, albeit indirectly, a series of biochemical reactions, including microsomal elongation, Δ-6 desaturation, and peroxisomal partial β-oxidation (13). These results showed that this pathway differs for the two fatty acid families: whereas no difference between the FSL and control rats was demonstrated for the n-3 PUFA family, the ratio for the n-6 PUFA family was lower in the FSL rats than in the controls.

DISCUSSION

The FSL rat model afforded us an exceptional opportunity to test the phospholipid hypothesis of depression in the brain tissue itself. Indeed, we found fatty acid alterations in selected areas of the brain, but these were somewhat unexpected, inasmuch as the main disturbance lay in the n-6 PUFA family and not the n-3 PUFA family. Specifically, the concentration of AA in brain tissue from various areas of the FSL rats was higher than in the corresponding brain areas in controls.

The possible involvement of AA in depression is being increasingly recognized because of the demonstrated effects of mood-regulating drugs on the turnover of this fatty acid. Studies have found that both chronic lithium chloride treatment (14) and chronic valproate treatment (15) decrease AA turnover in brain phospholipids. Moreover, some side effects of lithium chloride treatment could also be attributed to changes in AA signaling (16). Our study, however, is the first to show an actual disturbance in the level of a brain PUFA in an animal model of depression. The fact that the difference occurred in AA, the PUFA shown to be modulated by mood-stabilizing agents, adds further validity to our findings.

The present findings may also explain the beneficial effect of EPA supplementation in depressive patients (17). If, as in rats, the main PUFA disturbance in the brain of depressive patients is increased AA, it is possible that EPA treatment leads to a normalization of the increased level of AA rather than to an increase in the level of n-3 PUFA.

These results also suggest another hypothesis. It has recently been shown that under conditions of low brain DHA, this fatty acid is lost from the brain much more slowly than in controls (18). The opposite situation (i.e., that of increased brain PUFA) could be associated with a greater rate of loss. If this applied to the increased AA in the FSL rats, the activity of several metabolizing enzymes would have to be increased. Interestingly, the activity of some brain enzymes has been found to be decreased by mood-stabilizing drugs: phospholipase A_2 activity was downregulated by lithium chloride (19) and carbamazepine (20), and cyclooxygenase activity was downregulated by lithium chloride (21) and valproic acid (22).

Several studies in experimental animals have documented relationships between brain PUFA composition, including its dietary modification, and monoaminergic neurotransmission (23–25). However, all of them involved manipulations designed to change the n-3 PUFA composition of the brain in normal rats, based on findings that brain n-3 PUFA deficiency is associated with a variety of behavioral effects (26). In our study, both the FSL and the control rats were fed identical diets, suggesting that the differences in brain fatty acids might be related to the disease itself. Moreover, feeding an n-3 PUFA-deficient diet to rats did not cause alterations in the level of brain AA (18).

Our analysis of the 22 carbon chain n-6 PUFA (Table 2)

TABLE 3. Estimation of the final biosynthetic pathway for n-6 PUFA and n-3 PUFA

	Hypothalamus		Nucleus Accumbens		Prefrontal Cortex		Striatum	
Fatty Acid	Sprague-Dawley	FSL	Sprague-Dawley	FSL	Sprague-Dawley	FSL	Sprague-Dawley	FSL
22:5 n-6/22:4 n-6	0.14 ± 0.02	0.09 ± 0.01[a]	0.20 ± 0.04	0.12 ± 0.01[b]	0.24 ± 0.04	0.16 ± 0.01[c]	0.15 ± 0.01	0.12 ± 0.03
22:6 n-3/22:5 n-3	11.94 ± 3.15	9.14 ± 1.21	17.86 ± 4.26	17.59 ± 6.46	58.35 ± 18.72	72.50 ± 11.48	9.74 ± 2.68	9.95 ± 1.96

Values are means ± SD of the fatty acid ratios (n = 5).
[a] $P < 0.005$.
[b] $P < 0.05$.
[c] $P < 0.01$.

and of the final biosynthetic pathway of the two PUFA families (Table 3) suggests that certain processes exist in depressive rats that lead to the preferential accumulation of AA in the brain, without affecting n-3 PUFA metabolism. The possibility that desaturation and elongation of these two PUFA families could be differently regulated, especially under pathological conditions, has been discussed in detail (27). In this context, a separate regulation of phospholipase A_2 involved in the metabolism of n-3 and n-6 PUFAs has been shown in rat brain astrocytes (28).

We speculate that the derangement in brain neurotransmission that occurs in depression is associated with an increase in AA concentration, either by enhanced synthesis or decreased metabolism. Our results concerning the final metabolic pathways of n-6 PUFA (Table 3) favor the latter possibility, but any of the numerous potential mechanisms described for preferential AA retention in tissues may be operative (29).

It is unknown whether the increased brain AA demonstrated in our study results from the neuropathological processes involved in depression or the disturbed PUFA metabolism causes the manifestations of depression. Exploration of the effects of pharmacologic agents known to affect AA turnover (14, 15, 20) on the actual concentration of AA in the brain of depressive rats should help clarify this issue. Furthermore, detailed examination of the phospholipid species involved as well as the enzymatic pathways of PUFA metabolism in these rats can lead to new approaches toward understanding and treating this complex human disorder. Clearly, the FSL rat model of depression is a useful tool to elucidate the mechanisms underlying the fatty acid disturbances in this disease.

The authors thank Aviva Kluska for her expert technical work, Ilana Gelerentner for the statistical analysis, and Gloria Ginzach of the Editorial Board of Rabin Medical Center, for her help with the manuscript.

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