Effects of dietary n-3 or n-6 fatty acids on interleukin-1β-induced anxiety, stress, and inflammatory responses in rats

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Abstract **The present study demonstrated that an ω (n)-3 fatty acid, ethyl-eicosapentaenoic acid (ethyl-EPA), supplemented diet significantly attenuated the stress/anxiety behavior of rats in the "open field" and elevated plus maze, which was induced by subchronic intracerebroventricular administration of proinflammatory cytokine interleukin (IL)-1β. Ethyl-EPA also reduced the rise in serum corticosterone induced by IL-1. The n-6 fatty acid ethyl-γ-linolenic acid (ethyl-GLA) had little effect on the IL-1-induced changes in behavior and the corticosterone concentration. Following IL-1β administration, ethyl-EPA reduced the elevated prostaglandin (PG) E2 secretion and increased the secretion of antiinflammatory cytokine IL-10 from whole blood cells. Ethyl-GLA showed a similar antiinflammatory effect to ethyl-EPA. By contrast, n-6 fatty acid arachidonic acid (AA) had no effect on the behavior, immune, and endocrine changes induced by IL-1. AA alone enhanced the basal inflammatory response, raised serum corticosterone concentrations, and induced anxiety behavior in the elevated plus maze. The reduced growth rates of rats following the administration of IL-1 was attenuated by ethyl-EPA, and to a greater extent by ethyl-EPA plus ethyl-GLA, but not by AA alone or in combination with ethyl-EPA. Thus, ethyl-EPA would appear to antagonise the endocrine, immune, and behavioral effects of subchronic IL-1 administration. Ethyl-GLA only antagonised IL-1-induced inflammatory changes, whereas AA caused an increase in the secretion of corticosterone and PGE2, and induced anxiety-like behavior without enhancing the effects of IL-1.**—Song, C., X. Li, B. E. Leonard, and D. F. Horrobin. **Effects of dietary n-3 or n-6 fatty acids on interleukin-1β-induced anxiety, stress, and inflammatory responses in rats.** ***J. Lipid Res.*** **2003.** 44: **1984–1991.**

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Long-chain polyunsaturated fatty acids synthesized from dietary precursors such as α-linolenic and linoleic fatty acids are important components of membrane phospholipids in microglia, neurons, and immune cells (1, 2). Free fatty acids released into the blood, or passing through the blood-brain barrier, can act at specific binding sites such as the peroxisome proliferator-activated receptor, ion channels, or at allosteric sites on various proteins (1, 2). Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), synthesized from α-linolenic acids (18:3, n-3), and γ-linolenic acid (GLA) and arachidonic acid (AA) from linoleic acids (20:4, n-6), play major roles in membrane fluidity, lipid peroxidation, eicosanoid production, receptor and channel functions, and gene expressions (1–3). Changes in the phospholipid content of neuronal membranes can result in changes in signal transduction, neurotransmitter release, enzyme activity, and in neurotransmitter receptor and ion channel functions (1–3). Such changes have been implicated in the etiology of mood disorder and stress response in humans and behavioral changes in animals (1, 2, 4–6). In addition, changes in n-3 and n-6 concentrations have been linked to inflammatory and autoimmune diseases, including those affecting the brain, such as Alzheimer's disease and multiple sclerosis (7–10). These diseases are associated with inflammation in the brain and disturbances in brain function (11–13).

The phospholipid composition of cell membranes varies with their functions (14). The n-3 and n-6 fatty acids have been shown to fulfill different roles in the central nervous and immune systems (2, 15). The precursors of the n-6 and n-3 long chain fatty acids may only be converted slowly to their longer chain metabolites and may have actions that differ from their metabolites (3). For example, AA, an n-6 fatty acid released from membranes by the action of phospholipase (PL)A2, can be converted to proinflammatory

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Abbreviations: AA, arachidonic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; IL, interleukin; PGE2, prostaglandin E2.

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eicosanoids such as prostaglandin (PG)E2, leukotriene B4, and thromboxane A2. These three lipophilic molecules consequently induce inflammatory responses, such as fever, proinflammatory cytokine synthesis, and activation of phagocytosis, and induce cytotoxic changes (9, 16). By contrast, GLA, which is the precursor of AA, has been shown to inhibit inflammation (21). The n-3 fatty acids such as EPA, a precursor of DHA, reduce the synthesis of antibodies and proinflammatory cytokines, and suppress inflammatory responses by reducing membrane AA and eicosanoid synthesis (9, 17). A diet enriched with n-6 fatty acids has been shown to increase aggressive behavior in rodents, while one enriched with n-3 fatty acids reduced the stress response and improved learning and memory (18, 19). However, n-3 and n-6 fatty acids also interact with each other. For example, there is clinical evidence that ethyl-EPA improves symptoms of schizophrenia, an effect which may be related to an increase in AA synthesis (20), whereas a combination of EPA and GLA exhibits greater antiinflammatory effects than either GLA or EPA alone (21). Therefore, the interaction between n-3 and n-6 fatty acids at certain ratios would appear to be important for optimal membrane structure and functions and for normal signal transduction processes (1–3). There is, however, much to be learned about the relationship between the n-3 and n-6 fatty acids.

The inflammatory response initiated or attenuated by the polyunsaturated fatty acids is linked to the synthesis of the pro- or anti-inflammatory cytokine. Interleukin (IL)-1β, the most potent proinflammatory cytokine, has been found to induce stress and anxiety-like behavior in rodents (22–24). This cytokine stimulates the hypothalamus to release corticotropin-releasing factor (CRF), which, via adrenocorticotropic hormone, induces the secretion of glucocorticoids from the adrenals. IL-1β also activates central neurotransmitters, thereby increasing the turnover of noradrenaline, serotonin, and dopamine. The observed changes are similar to those seen when rodents respond to a stressor (23, 25).

Recent studies have demonstrated that lipopolysaccharide and IL-1β can induce the expression of β-amyloid protein and trigger microglia to produce proinflammatory cytokines and antibodies that result in brain inflammation (26, 27). Some of the effects of IL-1β on brain function are mediated by PGs, by activation of the PLA2-AA-cyclo-oxygenase (COX)2-PGE2 pathway during stress, and also following immune stimuli (28). An inhibitor of COX inhibitor has been reported to block the IL-1-induced elevation of corticosterone and also the behavioral response to pain (29). The evidence presented above suggests that a neuroinflammation may be causally related to mental disturbance such as stress and anxiety. Because n-3 and n-6 fatty acids can modulate both central nervous system function and inflammatory response, these questions arise: *1*) can single n-3 or n-6 fatty acids or a combination of both reverse changes induced by brain inflammation; and *2*) what are the mechanisms whereby the different types of fatty acids modulate the inflammatory changes? In the present study, the effect of diets enriched with different fatty acids and their combinations (ethyl-EPA, ethyl-GLA, AA, combination of EPA and GLA or EPA and AA) on anxiety, stress, and inflammatory response induced in rats by the central administration of IL-1β was evaluated.

MATERIALS AND METHODS

Animals and treatment

Male Wistar rats (initially weighing 200–220 g from Charles River, Quebec, Canada) were housed two per cage and maintained in a 12 h dark-light cycle at 21 ± 1°C. After habituation for 3 days, the rats were divided into 12 groups of 10 rats and fed with one of six different diets for 6 weeks; 5% palm oil was fed to rats as a control diet. The experimental diets were 4.5% palm oil mixed with 0.5% of each of fatty acid (ethyl-EPA, ethyl-GLA, or AA) and 4% palm oil mixed with a combination of 0.5% EPA and 0.5% GLA, or a combination of 0.5% EPA and 0.5% AA, respectively. The rats in each group were treated either with saline or 15 ng IL-1β by the intracerebroventricular route.

The body weights were determined twice weekly for the first 4 weeks during the presurgery period in which animals received the diets described above. Following the surgical insertion of the cannula for the intracerebroventricular administration, and following saline or IL-1 administration for 3 days, body weights were then measured daily.

Diets and preparation

The basal mix (Rx 991698 from Harlan Teklad Test Diet), palm oil (from Harlan Teklad Test Diet), and pure ethyl-EPA, ethyl-GLA, and AA oil (from Laxdale Ltd, UK) were stored at 4°C. The basal mix did not contain any fatty acids; 5% of the appropriate fatty acid mixture was added to 95% of the basal mix. The composition of diets is listed in **Table 1**. Palm oil was added to a beaker and then melted in a warm water bath (<50°C). The basal diet was then mixed with the palm oil followed by the addition of the appropriate concentration of the other fatty acids. The food was freshly prepared every 3 of 4 days and stored at 4°C.

Surgery

All rats were anesthetized with 100 mg/kg ketamine and 20 mg/kg xylazine. Tetracycline was used for treatment of the wound. A guide cannula was stereotaxically implanted at a position 1 mm posterior and 1.6 mm lateral of the bregma via a 1 mm-diameter burr hole. The guide cannula was inserted to 1 mm depth and secured to the skull with three screws using dental cement. A dummy cannula was then screwed into the guide cannula (30). The animals were allowed to recover for 14 days.

IL-1β and intracerebroventricular injection

Rat recombinant IL-1β was obtained from NIBSC, Potters Bar, UK (biological activity: 317 IU/mg) and dissolved in sterile, pyrogen-free saline at doses of 15 ng/10 μl/rat and prepared for intracerebroventricular administration.

TABLE 1. The nutrition composition in basal mix diet

Names	*g/kg*
Casein, "vitamin-free" test	202.11
DL-methionine	3.16
Sucrose	688.39
Cellulose	52.63
Mineral mix, AIN-93-MX (TD 94046)	36.85
Calcium phosphate, dibasic CaHPO4	3.69
Vitamin mix, AIN-93-VX (TD 94047)	10.53
Choline bitartrate	2.64

Rats were gently handled and held with a soft cotton towel daily for 2 weeks before the start of the intracerebroventricular injections. On the injection day, IL-1β or saline in a total 10 μl volume was taken into an internal needle (4.2 mm length) that was connected to a polyethylene 50 tube. After unscrewing the cap of a guide cannula, the needle was gently inserted into the guide cannula and IL-1 or saline was slowly infused into the brain over a period of 30 s. The injection needle was allowed to remain inside the guide cannula for 1 min and was then removed. Animals were returned to their cage after replacing the cap on the guide cannula. Rats were injected with saline or IL-1β every morning 50 min before behavioral testing (22).

Behavioral tests

The open field apparatus was made of aluminium and consisted of a white open circular area with a 90 cm diameter. A grid of 60 squares of 10 cm^2 was marked on the floor of the apparatus for quantifying locomotor activity. A 60 W bulb was positioned 90 cm above the center of the apparatus. Rats were placed singly in the center of the apparatus. The ambulation, rearing, grooming, and defecation scores, and the number of entries into the central zone of the apparatus, were recorded for a period of 3 min by means of a video camera (31). The apparatus was cleaned thoroughly with water after each animal had been tested.

The elevated plus maze consisted of an x-shaped maze elevated 1 m from the floor and comprised of two oppositely located enclosed and two open arms. The arms were 45 cm long and 10 cm wide. The rat was placed on the open central square formed by the arms at the start of each trial. The maze was lit by a 60 W bulb positioned in the center of the room. On the day of testing, each rat was placed singly on the central square of the maze, facing the open arm (32). The entries into open and closed arms and the time spent on these arms were recorded over a 5 min observation period. The arms were cleaned thoroughly with water after each test session.

Release of IL-10 and PGE2 from whole blood culture

Blood samples, taken by cardiac puncture under halothane anesthesia, were incubated with or without mitogens. The heparinized blood samples were diluted 1:10 with RPMI-1640 medium containing 1% penicillin, 5 μg/ml phytohaemagglutinin, and 20 μg/ml lipopolysaccharide. For a blank solution, the blood was diluted with RPMI-1640 containing 1% penicillin only. Samples (200 μl) were then pipetted into 24-well plates prefilled with medium (1,800 μl) and incubated for 72 h in a humidified atmosphere at 37°C, 5% CO_2. After incubation, the plates were centrifuged at 1,500 rpm for 15 min. The supernatant was removed under sterile conditions and frozen immediately at −70°C until the cytokine could be assayed (33).

The release of IL-10 and PGE2 was measured by a quantitative ELISA (Biosource International) and enzyme immunoassay (Assay Designs, Inc., Ann Arbor, MI), respectively, as described previously (33).

Measurement of corticosterone concentrations

Serum samples from trunk blood were used for the corticosterone measurement with a commercial radioimmunoassay kit (Immuchem corticosterone RIA kit for rats; catalog No. RCBK9906A; ICN Biochemical, Costa Mesa, CA). Intra- and inter-assay coeffients of variation were 6.8% and 5.6%, respectively.

Histological examination

After decapitation, brains were rapidly removed and placed on an ice block, and the locations of the cannulae and injection sites were quickly checked by slicing the brain coronally. Data were excluded from any animals in which the injection site failed to reach the lateral ventricle.

Statistical analysis

Results were analyzed by two-way ANOVA (IL-1 × diet) followed by Newman-Keuls post hoc for the comparison between two groups (**Table 2** and figures). The statistical package was obtained from GB-STAT, Dynamic Microsystems, Inc. Significance was set at a value of $P < 0.05$. Results are expressed as mean ± SEM.

RESULTS

EPA and GLA, alone and in combination, prevent the reduction of body weight after surgery or central IL-1β administration

There were no significant differences between the 12 groups in the gain of body weight after feeding with different diets for the first 4 weeks (5% of palm oil: 126 ± 7.07 g; 4.5% of palm oil and 0.5% of ethyl-EPA: 134.3 ± 6.43 g; 4.5% of palm oil and 0.5% ethyl-GLA: 128.58 ± 5.58 g; 4.5% of palm oil and 0.5% AA oil: 131.43 ± 6.82 g; 4% palm oil, 0.5% of ethyl-EPA, and 0.5% ethyl-GLA; 140.13 ± 7.33 g; 4% of palm oil, 0.5% of ethyl-EPA, and 0.5% AA oil: 128.18 ± 7.11 g). Three days following the implantation of the ventricular cannulae, the gain of body weight in the group fed with palm oil was significantly decreased (−4.44 ± 2.11 g) when compared with the weight gain before surgery (8.75 ± 3.36 g) ($P < 0.05$). This change was significantly attenuated in the group fed with the combination of ethyl-EPA and ethyl-GLA ($F_{5,115} = 3.89$, $P < 0.05$) (**Fig. 1A**). The gain of body weight was significantly reduced in palm oil-fed rats after intracerebroventricular IL-1β administration for 2 or 3 days ($F_{1,115} = 8.27$, $P < 0.01$) (data on day 2 not shown). When fed with ethyl-EPA alone, and in combination with ethyl-GLA, the weight reduction induced by central IL-1β administration was reversed ($F_{5,105} = 2.82$, $P < 0.05$) (Fig. 1B). Ethyl-EPA in combination with AA or any single fatty acid has no effect on the gain of body weight after surgery or IL-1 administration (Fig. 1).

TABLE 2. Effects of n-3 and n-6 fatty acids on animal behavior in "open field" following intracerebroventricular administration of IL-1β

	Locomotor	Rearings	Central Entries
5% Palm oil	132.0 ± 12.95	17.12 ± 1.3	1.6 ± 0.21
5% Palm oil + IL-1	76.37 ± 11.96[b]	8.0 ± 2.33[a]	0.1 ± 0.1[a]
0.5% EPA	106.8 ± 6.36	15.88 ± 1.37	1.5 ± 0.42
0.5% EPA+IL-1	112.33 ± 12.17[c]	15.13 ± 1.34[c]	1.44 ± 0.50[c]
0.5% GLA	121.4 ± 11.67	15.0 ± 2.76	1.2 ± 0.46
0.5% GLA+IL-1	108.7 ± 13.28	13.8 ± 1.95	0.4 ± 0.16
0.5% AA	149.88 ± 14.85	16.78 ± 1.49	1.6 ± 0.54
0.5% AA+IL-1	95.37 ± 16.82[a]	8.0 ± 1.98	0.78 ± 0.36
EPA+GLA	124.62 ± 4.58	14.25 ± 1.71	2.45 ± 0.35[d]
EPA+GLA+IL-1	99.0 ± 7.86	10.0 ± 1.68	0.5 ± 0.26[a]
EPA+AA	113.1 ± 6.68	14.6 ± 2.2	1.5 ± 0.34
EPA+AA+IL-1	93.5 ± 11.43	8.1 ± 2.19	0.8 ± 0.32

AA, arachidonic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; IL, interleukin. n = 8–11.

[a] $P < 0.05$.

[b] $P < 0.01$ versus the group with the same food + saline.

[c] $P < 0.05$ versus palm oil + IL-1 group.

[d] $P < 0.05$ versus GLA+saline.

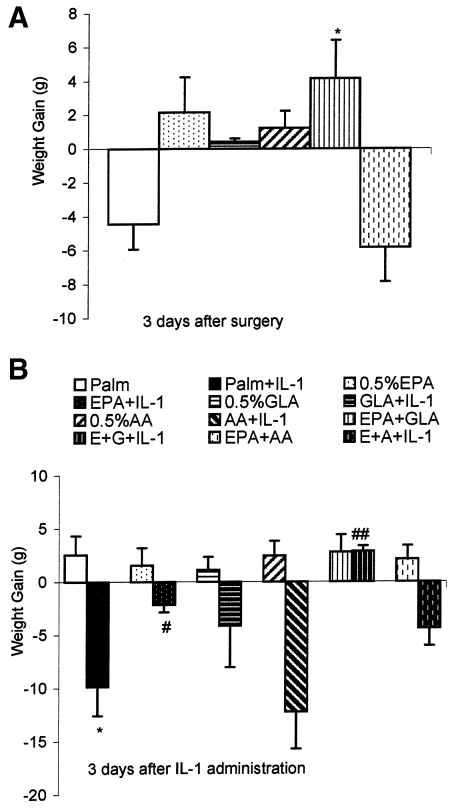

Fig. 1. The effect of diets enriched with n-3 and n-6 fatty acids on the gain of body weights 3 days after surgery or 3 days after interleukin (IL)-1β or saline administration. * $P < 0.05$ versus the palm oil with saline injection. # $P < 0.05$. ## $P < 0.01$ versus palm oil with IL-1β administration. n = 9–11. Error bars indicate SEM.

Effects of different diets on behaviors in the "open field" and elevated plus maze

Animals fed with different diets for 6 weeks did not show significant behavioral changes in the "open field" after intracerebroventricular saline administration. In the palm oil-fed group, intracerebroventricular IL-1β administration significantly reduced the locomotor activity (number of squares crossed), exploration (number of rears), and central zone entries (locomotor: F1, 115 = 3.56, $P < 0.05$; Rearing: F1, 115 = 3.33, $P < 0.05$; central zone: F1, 115 = 2.91, $P < 0.05$) (Table 2). ANOVA analysis indicated that IL-1-induced changes were attenuated by the n-3 and n-6 fatty acids (locomotor: F5, 105 = 7.94, $P < 0.0001$; Rearing: F5, 105 = 7.65, $P < 0.0001$; central zone: F5, 105 = 5.54, $P < 0.001$). The scores returned to control levels in animals fed with ethyl-EPA when compared with the scores in rats fed with palm oil diet and treated with IL-1β ($P < 0.05$). Ethyl-GLA feeding alone slightly attenuated the reduction in locomotor activity and the reduction in rearing scores in the rats treated with IL-1β ($P = 0.07$). The combination of ethyl-EPA together with GLA or AA, or AA alone did not significantly attenuate the effects of IL-1β (Table 2).

In the elevated plus maze, rats fed with AA oil and treated with saline showed a decrease in the ratio of the number of entries into open/closed arms when compared with the ratio in animals fed with palm oil ($P < 0.05$) (**Fig. 2A**). Rats fed with palm oil diets and treated with intracerebroventricular IL-1β significantly decreased the ratio of time spent in open/closed arms when compared with saline-treated rats fed with palm oil alone (entry number: F1, 115 = 5.98, $P < 0.01$; time spent: F1, 115 = 5.67, $P < 0.01$) (Fig. 2A, B). These anxiety-like changes in the elevated plus maze were markedly attenuated by ethyl-EPA treatment (ratio number: F5, 115 = 9.51, $P < 0.0001$; ratio time: F1, 105 = 5.18, $P < 0.001$). The other fatty acids, fed alone or in combination, did not prevent IL-1-induced changes (Fig. 2A, B).

Changes in serum concentrations of corticosterone after IL-1 administration in rats with different diets

Four of the five diets did not significantly change the serum concentrations of corticosterone following saline treatment. A small but significant increase in corticosterone was found in rats fed with AA oil ($P < 0.05$) (**Fig. 3**). A marked increase in corticosterone concentrations occurred in IL-1β-treated groups fed the supplemented diet with palm oil, ethyl-GLA, AA, or the combination of ethyl-EPA and ethyl-GLA (F1, 115 = 11.58, $P < 0.0001$) (Fig. 3). Only ethyl-EPA treatment alone significantly blocked the elevation of corticosterone induced by IL-1β administration ($P < 0.05$) (Fig. 3).

Modulation of different fatty acids on IL-1-induced inflammatory response

When comparing PGE2 release from the blood of animals fed with palm oil after saline treatment, intracerebroventricular saline treatment did not cause significant changes in the release of PGE2 or IL-10 in animals fed with

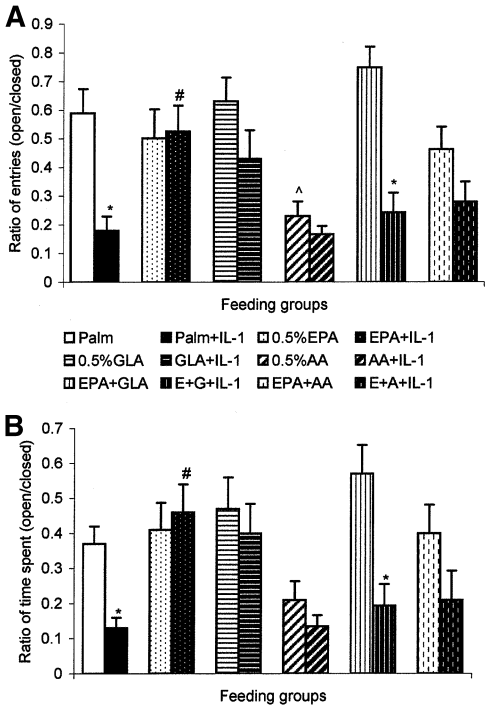

Fig. 2. The effect of diets enriched with n-3 and n-6 fatty acids on behavior in the elevated plus maze after intracerebroventricular IL-1β or saline administration. * $P < 0.05$ versus the group fed with same diet and with saline injection. # $P < 0.05$ versus Palm+IL-1β group. ^ $P < 0.05$ versus palm oil with saline. n = 9–11. Error bars indicate SEM.

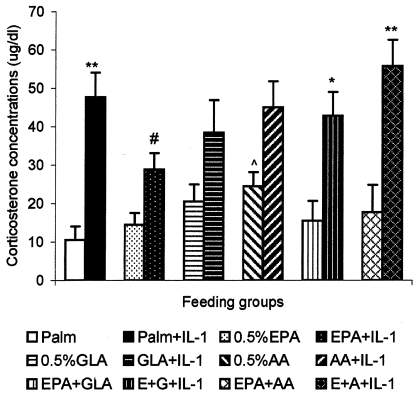

Fig. 3. The effect of diets enriched with n-3 and n-6 fatty acids on corticosterone secretion after intracerebroventricular IL-1β or saline administration. * $P < 0.05$. ** $P < 0.01$ versus the group fed with same diet treated with saline. # $P < 0.05$ versus the group fed with palm oil+IL-1β. ^ $P < 0.05$ versus palm oil with saline. n = 9–11. Error bars indicate SEM.

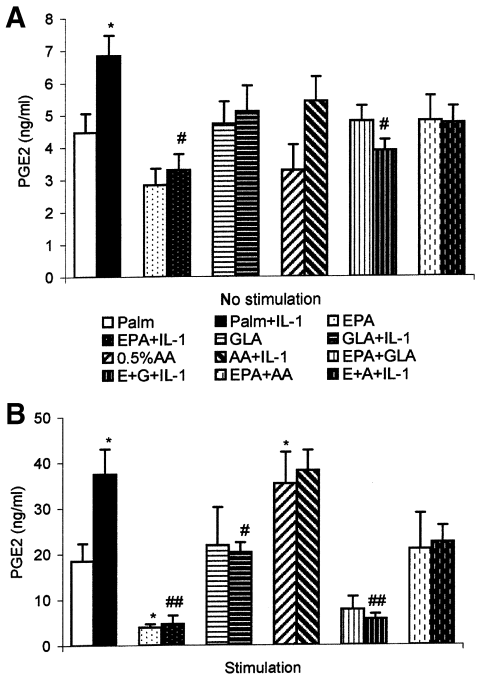

Fig. 4. The effect of diets enriched with n-3 and n-6 fatty acids on prostaglandin E2 release after intracerebroventricular IL-1β or saline administration. * $P < 0.05$ versus the group fed with palm oil and treated with saline. # $P < 0.5$. ## $P < 0.01$ versus palm oil with IL-1β administration. n = 9–11. Error bars indicate SEM.

ethyl-GLA or a combination of EPA and ethyl-GLA. The ethyl-EPA-supplemented diet suppressed PGE2 release from both nonstimulated and stimulated blood, while AA enhanced PGE2 release in stimulated blood ($P < 0.05$). In nonstimulated blood, central IL-1β administration significantly increased PGE2 release in animals fed with palm oil when compared with the saline-injected group on the palm oil diet (F1, 115 = 5.76, $P < 0.01$). PGE2 release was blocked by a diet enriched with ethyl-EPA, or a combination of ethyl-EPA and ethyl-GLA, but not by other fatty acids (F5, 115 = 4.29, $P < 0.01$). In mitogen-stimulated blood, IL-1β administration also induced a large increase in PGE2 release in animals fed with palm oil or AA oil (F1, 115 = 7.02, $P < 0.0001$). This elevation was prevented by ethyl-EPA, ethyl-GLA, or a combination of ethyl-EPA and ethyl-GLA (F5, 105 = 7.12, $P < 0.0001$) (**Fig. 4**).

IL-10 release from either nonstimulated or stimulated blood did not differ between the 12 groups following central saline injection. In nonstimulated blood, intracerebroventricular IL-1β administration induced a significant decrease in IL-10 release in most groups, with the exception of rats fed with a combination of ethyl-EPA and ethyl-GLA, in which IL-10 release was significantly higher than that in animals fed palm oil alone ($P < 0.05$). In mitogen-stimulated blood, IL-1β significantly suppressed IL-10 release in the palm oil fed groups (F1, 115 = 3.31, $P < 0.05$); whereas in animals fed with ethyl-EPA or ethyl-GLA, the reduction of IL-10 by IL-1 was significantly prevented (F5, 105 = 3.22, $P < 0.05$) (**Fig. 5**).

DISCUSSION

In the present study, the central administration of IL-1β significantly induced two types of changes. The first is an inflammatory-sickness response and the second a stress-anxiety-like response. The former response included lethargy (reduced locomotor activity), reduced body weight, enhanced PGE2 secretion, and suppressed release of anti-inflammatory cytokine IL-10. The latter response consisted of a decrease in the ratio of the number of entries into and time spent in the open/closed arms of the elevated plus maze, and a decrease in exploration and central zone entries in the "open field." The dramatic increase in serum corticosterone concentrations following IL-1β administration is also a reflection of a significant stress response. Diets supplemented with n-3 or n-6 fatty acids, or a combination of both exerted different effects on stress/anxiety-like behavior and inflammatory response, which is probably related to different roles that fatty acids play in the modulation of inflammatory response and glucocorticoid secretion.

The anti-inflammatory effects of n-3 fatty acid EPA have been widely studied. Dietary supplementation with EPA inhibits the production of proinflammatory cytokines and suppresses macrophage and other immune functions in both human subjects and laboratory animals (16, 34, 35). A fish oil-enriched diet has been shown to prevent weight loss and reduce the production of PGE2 and proinflammatory cytokines induced in rats by the systemic injection of lipopolysaccharide (36). Other investigators have shown that the anorectic effect of IL-1 is attenuated by n-3 fatty acids (52). In the present study, after surgery the reduction of weight gain was partially attenuated in the group fed the ethyl-EPA diet and significantly blocked in the group fed with the combination of ethyl-EPA and ethyl-GLA. The IL-1β-induced reduction in the growth rate was prevented in animals fed with ethyl-EPA or the combination of ethyl-EPA and GLA. The effect of the combination was more marked. These results indicated that a combina-

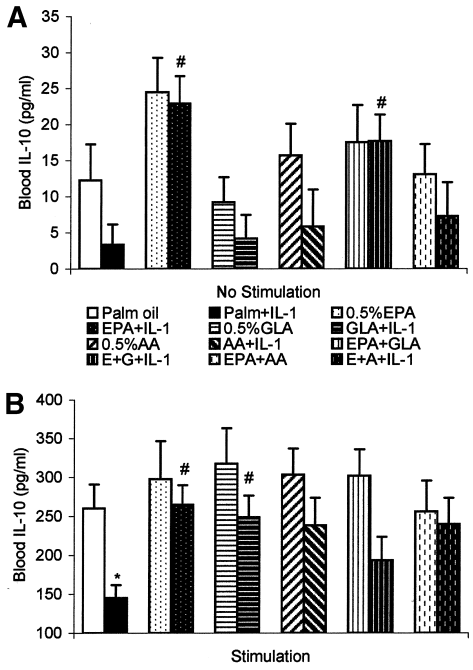

Fig. 5. The effect of diets enriched with n-3 and n-6 fatty acids on IL-10 release after intracerebroventricular IL-1β or saline administration. * $P < 0.05$ versus the palm with saline injection. # $P < 0.05$ versus palm oil with IL-1β administration. n = 9–11. Error bars indicate SEM.

tion of ethyl-EPA and ethyl-GLA has greater antiinflammatory effects than ethyl-EPA alone for physical recovery from injury and inflammation.

Previous studies revealed that a GLA-supplemented diet reduced the production of lipid mediators of inflammation and attenuated clinical symptoms of chronic inflammatory diseases (21, 37). Recently, GLA has been also found to reduce IL-1β release from monocytes (38). However, it is known that GLA increases AA synthesis (21) and that the excessive synthesis of AA may conversely lead to an increase in proinflammatory cytokines via the PLA2-COX2-PGE2 pathway (9, 16). Conversely, a combination of EPA and GLA treatment has been reported to suppress the generation of AA and the inflammatory mediator leukotrienes in the serum because EPA blocks 5-desaturase activity, the terminal enzymatic step in AA synthesis (21). In the present study, the ethyl-EPA and ethyl-GLA combination showed a greater effect than either ethyl-EPA or ethyl-GLA alone in attenuating the reduction of body weight following surgery or after IL-1β administration. However, neither the combination of ethyl-EPA and AA nor 0.5% GLA-supplemented diet significantly reverse this reduction in weight gain caused by IL-1.

The different effects of these fatty acids on body weight recovery or maintenance may be partially related to the effects of fatty acids on inflammatory changes. It has been shown in the present study that intracerebroventricular IL-1β administration induced a marked increase in the release of PGE2 and a decrease in IL-10 in nonstimulated blood (baseline condition) in rats fed palm oil. After feeding the rats with a diet enriched with ethyl-EPA or combination of EPA and GLA, these changes were attenuated. Diets enriched with AA, or a combination with ethyl-EPA, did not significantly prevent these inflammatory changes and also did not attenuate the IL-1-induced decrease in the body weight. Neither did the ethyl-GLA-supplemented diet, which reduced PGE2 and increased IL-10 release in mitogen-stimulated blood (antigen stimulated condition), significantly reverse the weight decrease induced by IL-1β. However, it was also noted that the antiinflammatory effect of ethyl-EPA was greater than the combination of EPA and GLA. Thus, there is a disparity between the effects of these fatty acids on the inflammatory response and the change in the body weight initiated by IL-1. Other mechanisms may be involved.

The "open field" apparatus is normally used to test animal response to a novel and stressful environment (31). In the "open field," central IL-1β administration significantly reduced locomotor, rearing, and central zone entries in the animals fed with the control diet of palm oil. In the elevated plus maze, a reduction of the ratio between number of entries into and time spent on open/closed arms was observed following IL-1β administration in animals fed with the control diet. In addition, the stress hormone corticosterone concentration was significantly increased following the cytokine infusion. These results suggest that IL-1 induced a stress and anxiety-like behavior. We and others have reported similar findings previously (22, 24, 25). The present study demonstrates that an ethyl-EPA-supplemented diet significantly attenuated the proinflammatory cytokine-induced stress and anxiety-like behavior. The evidence observed from the present study strongly suggests that the behavioral modulation of ethyl-EPA on both stress and anxiety-like behavior may be related to the reduction in the stress hormone corticosterone. Thus, ethyl-EPA significantly blocked the elevation of corticosterone levels induced by IL-1β and also normalized the behavior observed in both "open field" and elevated plus maze. Ethyl-GLA alone, or in combination with ethyl-EPA, did not prevent the increase in corticosterone levels or improve the behavior. Furthermore, the AA-supplemented diet increased corticosterone concentration and induced anxiety-like behavior (reduced the ratio of entry number into open/closed arms in the elevated plus maze). This may be explained by the fact that an increase in the release of CRF by IL-1 is dependent on the release of eicosanoids of n-6 series (53). Previous studies by others have reported that n-6-supplemented diet increased aggressive behavior in rats and that a diet enriched with AA oil increased corticosterone concentrations (18, 54). The n-6 fatty acid ethyl-GLA did not attenuate the stress response and corticosterone secretion, while n-3 fatty acids have been shown to reduce cardiovascular and adrenal responses following exposure to stress (39–42). In depressed patients, or in students exposed to psychological stress, EPA/AA was decreased and PGE2 was increased, which indicates that increased AA may play a role in stress or depression (5, 43, 44). The findings from the present study showed that AA increased corticosterone and PGE2, and decreased ratio number of entries into the open/closed arms, which was further support of the clinical findings.

The suppressive effect of ethyl-EPA on corticosterone cannot be separated from its anti-inflammatory effects on

PGE2. Among the three fatty acids, ethyl-EPA has the most potent antiinflammatory property (as observed in its effects on PGE2 and IL-10), which is significantly correlated with its suppression of corticosterone and normalization of stress and anxiety-like behavior. Ethyl-GLA alone, or in combination with EPA, has a mild antiinflammatory action, did not block the corticosterone elevation induced by IL-1, and only slightly reduced anxiety-like behavior. Conversely, AA increased PGE2 and corticosterone and increased anxiety-like behavior. IL-1β induces changes in the inflammatory response and corticosterone secretion through the activation of PGE2 and its receptor (29, 45, 46). This cytokine also markedly increases gene expression of the PGE2 receptor in the several brain regions (47). The mechanism by which ethyl-EPA suppresses PGE2 may be via the inhibition of eicosanoid synthesis (9, 16, 34), whereas the reduction in corticosterone secretion following stress by the combination of EPA and GLA could arise from a reduction in the availability of cholesterol, the precursor of corticosterone (48). The lack of effect of ethyl-GLA alone or in combination with ethyl-EPA on the behavior may be explained by the GLA induced synthesis of AA that results in increases in corticosterone and PGE2 (9, 54).

In a previous study, we found that 0.2% EPA partially but significantly reversed and 1% EPA completely reversed some changes induced by IL-1β (50). Little is known about the optimal dose of GLA that modulates inflammatory processes in the brain. In addition, there appears to be no consistent information on n-3 and n-6 ratios used by other investigators. Several studies reported that optimal ratio of n-3 to n-6 is 1:4 (49). The reason that we chose a 1:1 ratio in the present study is based on the following evidence: *1*) in a pilot experiment, the ratio 1:4 (EPA- GLA) did not reverse IL-1 induced changes. *2*) We have previously reported that soy bean oil in which the ratio of n-3 to n-6 is 1:7 cannot reverse IL-1-induced changes (50, 51). *3*) Others have reported that when n-6 concentration in the brain is high, a much higher ratio between n-3 and n-6 (1:1 or 2:1) was needed to reverse abnormal behavioral induced by n-3 deficiency (19). *4*) After central IL-1β infusion, inflammation may increase n-6 synthesis and metabolism. However, in the present study, even a ratio of EPA-GLA of 1:1 did not significantly attenuate some of behavioral and inflammatory changes. In future studies, a higher dose of ethyl-GLA, and different ratios between EPA and GLA or AA, shall be studied.

In summary, the present study demonstrated that n-3 fatty acid ethyl-EPA at 0.5% of total dietary fat significantly reversed central IL-1β-induced stress and anxiety-like behavior, stress hormone secretion, and inflammatory responses in both nonstimulated and stimulated blood. An ethyl-GLA-supplemented diet at the same dose significantly blocked increased PGE2 and decreased IL-10 induced by IL-1β in mitogen-stimulated blood but did not significantly attenuate the elevated corticosterone and stress and anxiety-like behavior. A 0.5% AA supplemented diet significantly increased PGE2 and corticosterone secretion, and induced anxiety-like behavior in the elevated plus maze. This n-6 fatty acid lacked an effect on IL-1-induced changes. The combination of ethyl-EPA and ethyl-GLA synergistically prevent the reduction of body weight after surgery or IL-1β administration and corrected some inflammatory changes, but did not significantly affect the corticosterone and behavior induced by IL-1β. The combination of ethyl-EPA and AA did not affect any of the changes induced by IL-1β. These results indicate that among three different fatty acids at 0.5% concentration, EPA treatment alone is more effective in the modulation of stress hormone corticosterone, stress, and anxiety-like behavior, which were increased by IL-1β. The range of antiinflammatory effects of these fatty acids on IL-1-induced changes in the release of PGE2 and IL-10 appeared as ethyl-EPA > ethyl-EPA + GLA > ethyl-GLA > AA. However, the combination of EPA and GLA was more effective for body weight recovery following a surgery and IL-1 administration.

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