Indian Journal of Biochemistry & Biophysics
Vol. 38, October 2001, pp. 327-330

(50)

Polyunsaturated fatty acids (PUFA) regulate neurotransmitter contents in rat brain

Sheelu Varghese[1], Bake Shameena[1], Lakshmy P S[1], Biju M P[2], Easwar Shankar P N[2], Paulose C S[2] and Oommen V Oommen[1]*

[1] Department of Zoology, University of Kerala, Kariavattom, Thiruvanantpuram 695 581, India
[2] Department of Biotechnology, Cochin University of Science and Technology, Cochin 682 022, India

Received 29 May 2000; revised and accepted 27 August 2001

The effects of feeding of 6-propylthiouracil (6-PTU) and polyunsaturated fatty acids (PUFA) independently and in combination and administration (*ip*) of a single dose of triiodothyronine (T_3) (2.5μg/100g body wt) along with feeding of 6-PTU and PUFA were studied in rat brain. Dopamine (DA), 5-hydroxytryptophan (5-HTP), serotonin (5-HT), 5-hydroxy indole acetic acid (5-HIAA), norepinephrine (NE) and epinephrine (EPI) contents were assayed in the hypothalamus and cerebral cortex regions. It was found that 6-PTU feeding resulted in decrease in dopamine, 5-HT, 5-HTP and 5-HIAA in both regions. In animals fed with PUFA followed by administration of T_3, the DA level was found normal.

In vertebrates, hypothyroidism and hyperthyroidism cause abnormalities of cognitive function. Congenital hypothyroidism is accompanied by morphological abnormalities of the cerebral cortex and cerebellum. It was reported that thyroid hormones mobilize the brain tissues during differentiation and T_3 was abundantly required for the synaptome functions[1]. However, quantitatively and qualitatively distinct patterns of thyroid hormone metabolism exist in different regions of brain[2,3]. Hypothyroidism leads to marked neurological manifestations like mental disorder, emotional instability and many disorders like Parkinsonian symptoms[4]. It was reported that cerebral cortex and hypothalamus have high affinity T_3 binding sites in higher concentration compared to other areas of brain[5].

Neuronal membranes of the brain constitute high amount of polyunsaturated fatty acids (PUFA). About 20% of the dry weight of the brain constitute essential fatty acids. Hence any change in the relative content of fatty acids may affect cognitive function and behaviour[6-8]. Fatty acids incorporated into phospholipids are very important in maintaining the structural and functional integrity of neuronal membrane[9]. Recently it was reported that not only the level of essential fatty acids but also the ratio between the n-3 and n-6 fatty acids is critical in mediating cognitive and biochemical functions[10]. Fish oil rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) induced changes in brain PUFA composition characterised by high levels of EPA and DHA and compensated for low levels of arachidonic acid (C20:4 n-6). High dietary fish oil during fetal period in rat modified brain fatty acid composition and also improved learning ability[11]. Change in the physical state of the membrane can affect the membrane receptors. Essential fatty acids modify the functions of neurotransmitter receptors such as cholinergic, muscarinic, nicotinic, adrenergic, N-methyl-D-aspartate (NMDA) and dopaminergic receptors[10].

In this paper, we report the effect of T_3 and PUFA on the concentration of various neurotransmitters and their metabolites, such as NE, EPI, 5-HTP, DA, 5-HT and 5-HIAA in the hypothalamus and cerebral cortex of male Wistar rats.

Materials and Methods

Diet and hormone treatment

Male Wistar strain of rats weighing ~200 g (Penpol, Trivandrum, India) were used for this study. The animals were maintained at room temperature (25±2°C) and subjected to natural photoperiod. After rats were fed on a basal diet for two weeks, those without any abnormality in growth were divided into five groups, each containing six animals.

The basal diet consisted of glucose 60%, casein 24%, cellulose powder 10%, salt mix 4% and vitamin mixture 1% supplemented with 1% sunflower oil as a source of essential fatty acids. Group 1 received basal feed and served as control. Group 2 received 6-

*Author for correspondence.
E-mail: oommen@md2.vsnl.net.in

Abbreviations used : T_3, triiodothyronine; PUFA, polyunsaturated fatty acid; NE, norepinephrine; EPI, epinephrine; 5-HTP, 5-hydroxy tryptophan; 5-HT, Serotonin; 5-HIAA, 5-hydroxyindole acetic acid; DA, dopamine.

propylthiouracil (6-PTU) administered as 0.1% solution in drinking water for 20 days and fed with basal feed. Group 3 rats received same dose of 6-PTU and were fed with basal feed with an additional supplementation of 10% (v/w) cod liver oil for 20 days. Group 4 rats were fed with basal feed with an additional supplementation of 10% cod liver oil for 20 days while those of group 5 animals were fed with basal feed containing 10% cod liver oil + 6-PTU for 20-days + single intraperitonial injection of triiodothyronine (T_3) 2.5μg/100g body weight. The dose of 6-PTU and T_3 selected here are reported to produce a metabolic change in rat liver[12]. All animals were sacrificed 24 hr after final injection/feeding and were kept deprived of food 24 hr before sampling. Rats were sacrificed by decapitation. The brain was rapidly ,sected into different regions[13]. The dissection was carried out on a chilled glass plate. Hypothalamus and cerebral cortex were frozen in liquid nitrogen until analyzed.

Neurotransmitter assay

Brain neurotransmitters were assayed using High Performance Liquid Chromatography (HPLC). DA, NE, EPI, 5-HTP, 5-HIAA and 5-HT were assayed according to the procedure described earlier[14]. A 10% homogenate of the hypothalamus and cerebral cortex was prepared in 0.4 *N* perchloric acid. The homogenate was centrifuged at 5000 × *g* for 5 min at 4°C. The supernatant was filtered through 0.45 μm syringe top filters (Millipore). The filtered sample (20 μl) was injected into a HPLC system (Shimadzu, Japan) with C-18 reverse phase column. The mobile phase consted of 75 m*M* sodium phosphate monobasic, 1 m*M* sodium octyl sulphonate, 50 m*M* EDTA and 7% acetonitrile. The *p*H was adjusted to 3.45 with phosphoric acid. A flow rate of 1 ml/min was maintained with Shimadzu solvent delivery module. The neurotransmitters and their metabolites were detected using an electrochemical detector (model 6A, Shimadzu, Japan) with a reduction potential of 0.8 V. The peaks were identified by relative retention times compared with standards and quantitatively estimated using an integrator interfaced with the detector.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) and significant difference between the groups was determined by Duncan's multiple range test[15] at the level $p<0.05$.

Results

It is seen from Table 1 that dopamine concentration decreased significantly in the hypothalamus of 6-PTU treated rats whereas it was normal in rats treated with PUFA and 6-PTU + PUFA+T_3. High DA content was observed in rats fed PUFA alone. Similarly, in the cerebral cortex also there was decrease in DA contents in the hypothyroid rats and normal DA content was observed in rats treated with PUFA (Table 2). A combination of PUFA and T3 administration resulted in normal DA content in the hypothalamus and cerebral cortex of 6-PTU treated rats (Tables 1 & 2). In the case of 5-HT, the treatment caused an increased turn over of 5-HT to 5-HIAA leading to a decreased accumulation of 5-HT in the hypothalamus. Norepinephrine level was unaltered by either of the treatment. However, epinephrine was increased in all treated groups (Table 1).

Table 1—Neurotransmitter content (n moles/mg wet wt. tissue) in the hypothalamus of rats
[The different diets and administration of T3 to rats are explained under Materials and Methods]

Neurotransmitters	Control	6-PTU	6-PTU+PUFA	PUFA	6-PTU+PUFA+T_3
DA	0.078 ± 0.01^{a}	0.03 ± 0.009^{b}	0.09 ± 0.02^{a}	4.79 ± 1.98^{c}	0.07 ± 0.01^{a}
5-HTP	0.8 ± 0.09^{a}	0.31 ± 0.07^{b}	2.8 ± 0.58^{c}	0.5 ± 0.15^{a}	0.65 ± 0.08^{a}
5-HT	0.05 ± 0.002^{a}	0.02 ± 0.003^{b}	0.05 ± 0.02^{ac}	0.08 ± 0.003^{ac}	0.02 ± 0.004^{a}
5-HIAA	3.5 ± 0.15^{a}	2.46 ± 0.52^{b}	5.94 ± 0.66^{c}	2.89 ± 0.29^{ab}	2.87 ± 0.82^{ab}
NE	51.35 ± 5.6^{a}	57.56 ± 17.6^{a}	58.2 ± 7.7^{a}	55.3 ± 5.3^{a}	57.4 ± 8.7^{a}
EPI	13.3 ± 4.0^{a}	80.24 ± 8.2^{b}	104.5 ± 43.8^{b}	111.2 ± 18.26^{b}	103.3 ± 9.5^{b}

Results are expressed as mean ± SD of five samples (n=5). Mean values with different superscript letters are significantly different within a row when analysed by one way ANOVA with Duncan's Multiple Range Test at the level of $p<0.05$

Table 2—Neurotransmitter content (n moles/mg wet wt. tissue) in the cerebral cortex of rat
[The different diets and administration of T3 to rats are explained under Materials and Methods]

Neurotransmitters	Control	6-PTU	6-PTU+PUFA	PUFA	6-PTU+PUFA+T_3
DA	0.27 ± 0.02^{a}	0.12 ± 0.01^{b}	0.33 ± 0.15^{a}	0.54 ± 0.14^{c}	0.38 ± 0.14^{a}
5-HTP	0.67 ± 0.05^{a}	0.08 ± 0.03^{b}	0.22 ± 0.15^{c}	0.81 ± 0.08^{d}	0.64 ± 0.05^{a}
5-HT	0.14 ± 0.05^{a}	0.14 ± 0.008^{a}	0.13 ± 0.04^{a}	0.25 ± 0.03^{b}	0.13 ± 0.01^{a}
5-HIAA	2.59 ± 0.44^{a}	3.44 ± 0.63^{a}	2.84 ± 0.76^{a}	2.97 ± 0.91^{a}	2.46 ± 0.5^{a}
NE	48.03 ± 10.3^{a}	32.2 ± 8.1^{a}	33.08 ± 11.9^{a}	58.96 ± 24.1^{ab}	33.48 ± 9.0^{a}
EPI	2.81 ± 0.5^{a}	2.2 ± 0.1^{a}	3.88 ± 2.78^{a}	3.67 ± 0.44^{a}	4.15 ± 0.19^{ac}

Results are expressed as mean ± SD of five samples (n=5). Mean values with different superscript letters are significantly different within a row when analysed by one way ANOVA with Duncan's Multiple Range Test at the level of $p<0.05$

Dopamine content was low in 6-PTU treated animals and a high concentration was observed in PUFA treated group in the cerebral cortex. In all other experimental animals no significant change was observed. Serotonin content in the cerebral cortex was unaffected by any treatment except in PUFA treated group where as an increased 5-HT level was found. However, 5-HIAA concentration was not affected by either of treatment. NE or EPI level was not altered by any of the treatments (Table 2).

Discussion

PUFA, an essential membrane component acts as a modulator for many enzymatic reactions. Thyroid hormones are essential regulatory factors in neuronal migration and many cognitive functions[16,17]. In this study, rats were made hypothyroidic by feeding antithyroid drug 6-PTU and T_3 was injected to hypothyroid animals. In order to alter fatty acid composition, a diet rich in n-3 and n-6 PUFAs was given by including cod liver oil. With this diet, the supply of PUFA would be greater than minimal requirement for adequate PUFA levels in the membrane. Very little information is available on the proportion of different lipid classes and their effects on brain membranes and functions.

Cod liver oil enriched diet may modify the fatty acid composition of rat brain and may influence neurochemical and behavioural aspects of monoaminergic functions. The significant reduction observed in the DA concentration in the hypothalamus and cerebral cortex of 6-PTU treated rats may be due to the antagonistic effect of TSH which increases in hypothyroid condition. It has been reported that TSH release is controlled by a dopaminergic mechanism in man. The more accentuated TSH response in hypothyroid patients may perhaps be attributable to the absence of a negative feed back by thyroid hormones[18]. The decrease in DA content caused by 6-PTU is overcome in 6-PTU+PUFA and 6-PTU+PUFA+T_3 treated animals. However, a high DA content was present in PUFA treated animals. Increased dopamine concentration was reported in rats fed with polyunsaturated fatty acid[19]. In another report, a marked decrease was suggested in endogenous dopamine content in rats fed with a PUFA deficient diet[20]. An increased concentration of dopamine is observed in T_3 treated groups compared to 6-PTU treated animals in this study. This is possibly caused by PUFA or the action of T_3 through decreased TSH level.

Variations in brain monoaminergic neurotransmission system have been reported to occur in human and in experimental animals as a consequence of ageing or neuro disorders such as Alzheimer's disease, Schizophrenia and Parkinsonism[21]. Hypothalamic serotonergic system is involved in the regulation of food ingestion and energy metabolism. However, the exact role of PUFA on serotonin turn over is not understood. It was reported that diet increases 5-HT release in experimental animals[22]. In our experiment hypothalamic 5-HT content is not altered by PUFA ingestion. However, an increased 5-HT content was observed in cerebral cortex with a high concentration of 5-HTP. 5-HIAA is not increased significantly resulting in the accumulation of 5-HT content. This result suggests that cerebral cortex is more responsive to PUFA treatment. It was reported that serotonin con-

centration was not affected by PUFA treatment in the frontal cortex[23]. It is evident that a direct relationship exists between serotonin turnover and thyroid stimulating hormone (TSH) release[24].

Among the major factors that regulate TSH level in the body is circulating T_3 level. In 6-PTU treated animals, hypothyroidism leads to increased TSH level. In our study, 5-HT content decreased in hypothyroid animals with a decreased concentration of 5-HTP and 5-HIAA in hypothalamus. However, in cerebral cortex no such change was observed. This may be due to the action of high levels of TSH, because both are antagonistic in action. It was reported that hypothalamic type of hypothyroidism results in decreased hypothalamic serotonin level[25]. However, when hypothyroid animals were treated with PUFA the effect of 6-PTU on serotonin level was reversed with an increased 5-HTP and 5-HIAA levels. This effect is probably due to the action of PUFA on serotonin level in hypothalamus. In T_3 treated animals there is no change in 5-HT, 5-HTP and 5-HIAA content in hypothalamus and cerebral cortex.

It is well documented that dopaminergic and serotoninergic receptors are affected differentially by the diet. These two monoaminergic systems interact with each other, and in particular 5-HT is a tonic inhibitor on DA function in physiological conditions. Hence, it is difficult to ascertain the exact role of the two monoaminergic neurotransmitters under the present experimental conditions. EPI content is high in all treated groups suggesting an increased biosynthesis of EPI from NE. However, in cerebral cortex no such change is observed. The feeding of fish with dietary fat rich in (n-6)/ (n-3) polyunsaturated fatty acids affected monoaminergic neurotransmission and behaviour in rats, and our results correlate with these evidences and a deficiency in polyunsaturated fatty acids is reported to cause a decrease in neurotransmitter contents that could be involved in behavioural abnormalities [19].

Acknowledgement

We are grateful to Mr Kalyanaraman, Department of Statistics, University of Kerala for statistical analysis and interpretation of data.

References

1 Bernal J & Nunez J (1999) *Eur J Endocrinol* 13, 390-398
2 Kaplan M M & Yaskoski K A (1980) *J Clin Invest* 66, 551-562
3 Farwell A P & Duboard-Tomasetti S A (1999) *Edocrinol* 140, 4221-4227
4 Ray A K & Sarkar P K (1997) in *Frontiers in Environmental and Metabolic Endocrinology* (Maitra S K ed.) pp. 229-239, The University of Burdwan, India
5 Mahio Y, Inada M, Tanaka K, Ishii H, Naito K, Nishikawa M, Takahashi K & Imura H (1983) *Acta Endocrinol (Copenh)* 104, 134-138
6 Yamamoto N, Saitoh M, Moriuchi A, Nomura M & Okuyama H (1987) *J Lipid Res* 28, 144-151
7 Bourre J M, Francois M, Youyou A, Dumont O, Piciotti M, Pascal G & Durand G (1989) *J Nutr* 119, 1880-1892
8 Yahuda S, Rabinovitz S, Mostofsky D I, Huberman M, Carasso R L & Srednic B (1997) *Euro J Pharmacol* 328, 23-29
9 Bourre J M & Dumont O (1991) *World Review of Nutrition and Dietetics* 66, 103-117
10 Yehuda S, Rabinovitz S & Mostofsky D I (1997) in *Hand Book of Essential Fatty Acids Biology* (Yehuda S, Mostofsky D I eds.) pp. 427-452, Humana Press, New Jersey
11 Yonekubo A, Honda S, Okano M, Takahashi K & Yamamoto Y (1994) *Biosci Biotech Biochem* 58, 799-801
12 Lanni A, Moreno M, Cioffi M & Goglia F (1992) *Mol Cell Endocrinol* 86, 143-148
13 Glowinski J & Iversen L L (1966) *J Neurochem* 13, 655-669
14 Paulose C S, Dakshinamurti K, Packer S & Stepehns N L (1988) *Hypertension* 11, 387-391
15 Duncan D B (1955) *Biometr* 11, 1-42
16 Angulop-Guerrero O & Oliart R R (1998) *Arch Latinoam Nutr* 48, 293-298
17 Farwell A P & Duboard-Tomasetti S A (1999) *Endocrinol* 140, 4221-4227
18 Massara F, Cammani F, Belforte L, Vergano V, Molinatti GM (1978) *Clin Endocrinol* 9, 419-428.
19 Chalon S, Delion Vancassel S, Belzung C, Guilloteau D, Leguisquet A M, Besnard J C & Durand G (1998) *J Nutr* 128, 2512 - 2519
20 Delion S, Chalon S, Guilloteau D, Besnard J C & Durand G (1996) *J Neurochem* 66, 1582-1591
21 Van Kammen & Gelernter A (1987) in *Psychopharmacology: The Third Generation of Progress* (Meltzer H Y ed) pp. 753-758, Raven Press, New York
22 Mori R C, Guimaraes R B, Nascimento C M & Ribeiro E B (1999) *Can J Physiol Pharmacol* 77, 286-292
23 Delion S, Chalon S, Guilloteau D, Lejeune B, Besnard J C & Durand G (1997) *J Lipid Res* 38, 680-689
24 Dakshinamurti K, Paulose C S & Vriend J (1985) *J Endocrinol* 109, 345-349
25 Dakshinamurti K, Paulose C S & Viswanathan M (1990) *Annals N Y Acad Sci* 585, 241-249