Chronic n-3 polyunsaturated fatty acid diet-deficiency acts on dopamine metabolism in the rat frontal cortex: a microdialysis study

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Received 14 October 1997; received in revised form 5 December 1997; accepted 10 December 1997

Abstract

The effects of \(\alpha\)-linolenic acid diet deficiency on rat dopaminergic metabolism were investigated in the frontal cortex of male 2–3 month-old rats using the microdialysis method. Increased basal levels of dopamine metabolites were observed in the frontal cortex of awake deficient rats, without modification of dopamine levels. Moreover, using KCl perfusion which releases newly synthesized dopamine, no difference was observed in anaesthetized deficient rats versus control rats. In addition, a decrease in dopamine release was observed in anaesthetized deficient rats versus control rats after tyramine stimulation, which is known to induce release of dopamine from vesicular stores. A working model is proposed which suggests that a chronic n-3 polyunsaturated fatty acids (PUFA) deficiency may lead to modifications in the internalization of dopamine in the storage pool in the frontal cortex.

Keywords: Dopamine; Dopamine metabolites; Frontal cortex; n-3 Polyunsaturated fatty acid; Microdialysis; Tyramine

Polyunsaturated fatty acids (PUFA) are essential components of the structural membrane lipids. They are actively accumulated in the brain during development, are involved in the formation of new cell membranes in neuronal tissue [15] and are major constituents of synaptic vesicles [12]. Two families of PUFA, essential because not synthesized by mammals, are distinguished by their precursors: linoleic acid for the (n-6) series, and \(\alpha\)-linolenic acid for the (n-3) series. It is known that a diet deficient in \(\alpha\)-linolenic acid greatly affects the fatty acid composition of membrane phospholipids in the central nervous system [1,17]. These changes include alterations in membrane architecture and fluidity [11], enzyme activity and membrane electrophysiology [2]. In addition, previous findings in our laboratory suggest that the dopamine neurotransmission system is affected by n-3 PUFA deficiency: rats with long-term deficiency showed decreased levels in endogenous dopamine and decreased D2 receptor binding in the frontal cortex but not in the striatum or cerebellum [4,5]. Moreover, behavioural effects of n-3 PUFA deficiency in rodents were indicative of changes in attention, motivation, and reactivity to stimuli and rewards, whereas locomotion remained unchanged [7,16]. These effects might be consistent with a deficit in the function of the prefrontal dopamine pathways [3,14]. However, the biochemical mechanisms of this dys-function still remain to be clarified. The aim of this work was therefore a better understanding of these mechanisms, and then we opted for a dynamic approach to neurotransmission processes using the microdialysis method. We studied basal dopamine release in awake rats using a physiological approach, and stimulated release in anaesthetized rats using a pharmacological approach. We chose pharmacological stimulation in anaesthetized animals because massive dopamine release in the cortical area causes major convulsions in awake animals. Moreover, anaesthesia frees the frontal cortex from external stimuli which could modify cortical dopaminergic release.
Two generations of female Wistar rats originating from the Laboratoire de Nutrition et Sécurité Alimentaire, INRA, were fed a diet containing 6% fat in the form of African peanut oil specifically deficient in $\omega$-linolenic acid as already described [4]. This deficient diet provided 1200 mg of linoleic acid but less than 6 mg of $\omega$-linolenic acid per 100 g of diet. Two weeks before mating, female rats originating from the second generation were divided into two groups. The first group received the deficient diet, and the second group received a diet in which peanut oil was replaced by a mixture of 60% peanut oil and 40% rapeseed oil. This diet (control) provided the same amount of linoleic acid as the deficient diet and in addition 200 mg of $\omega$-linolenic acid per 100 g of diet ($[\omega-6]/[\omega-3] = 6$). Diets were consumed ad libitum by both groups. At weaning, the male progeny of these two groups of female rats received the same diets as their respective dams. When the male rats reached 250–300 g (2–3 months of age) they were used for the microdialysis studies. The animals were placed in a stereotaxic apparatus (Stoelting, USA) under ketamin (Imalgene, Rhône Mérieux, France) anesthesia (1.5 mg/kg i.p. and then i.m. if necessary). The probe guide (CMA/Microdialysis, Sweden) was implanted in the left medial frontal cortex (coordinates A-P: 3.2; M-L: 1.2; D-V: -6, from Bregma) according to [13]. After fixation of the guide, a microdialysis probe with 10% recovery (polycarbonate, 15 kDA cut-off, 4 mm length) (CMA 12; CMA/Microdialysis, Sweden) was slowly lowered into the brain. The probe was continuously perfused with buffer (Dulbecco modified liquid; ICN, USA) at 0.8 ml/min using a microsyringe pump (Harvard Apparatus, USA). Body temperature was maintained at 37 ± 1°C using a thermostatically controlled heating blanket (CMA 150; CMA/Microdialysis Sweden). After pre-perfusion for 1 h, 3–4 20 min dialysate samples were collected from the frontal cortex and were defined as basal level before drug perfusion. Potassium chloride (KCl 200 mM; Sigma, USA) or tyramine (200 μM; RBI, USA) was perfused in situ into the brain using a microinfusion pump (CMA 12; CMA/Microdialysis, Sweden) at a rate of 5 μl/min. Body temperature was maintained at 37 ± 1°C using a thermostatically controlled heating blanket. The perfusate volumes were 20 min fractions (100 μl) and kept at –80°C until analysis. For microdialysis on anaesthetized animals, rats under urethane anaesthesia (Sigma, USA) at a dose of 1.5 g/kg i.p. were placed in the stereotaxic apparatus. The microdialysis probe (without guide) was then implanted in the left medial frontal cortex as in the previous experiment, and continuously perfused with the perfusion buffer at 5 μl/min.
performed with a 5 ml/min (Gold 118 system; Beckman, USA). Separation was achieved by a pentobarbital bolus (Sanofi, France) and the probe site was macroscopically examined on sections. The right cortex was dissected, prepared and kept at −80°C until dopamine levels were measured, according to previous studies [4]. Dialysate levels of catecholamines were measured using HPLC with electrochemical detection. An isocratic mobile phase (7% acetonitrile, 3% methanol, 90% of citric acid 0.02 M plus NaH₂PO₄ 0.01 M, octanesulfonic acid 0.0045 M, heptanesulfonic acid 0.003 M, EDTA 0.0001 M, o-phosphoric acid and diethylamine, pH 2.5) was pumped at 0.3 ml/min (Gold 118 system; Beckman, USA). Separation was performed with a 5 μm C18, 3.2 × 100 mm column (LC-22C; BAS, USA). A glassy carbon working set at 0.8 V with reference to an Ag/AgCl electrode was used to detect compounds of interest.

The results between groups of deficient and control rats were compared by one-way analysis of variance (ANOVA; diet-factor). Dopamine values were subjected to a logarithmic transformation to achieve homogeneity of variance. The results between the different groups were tested using the t-test for unpaired values. For both tests values were considered significantly different when P < 0.05. The endogenous dopamine level in the frontal cortex was 34% lower in deficient rats compared to control rats (0.12 ± 0.01 versus 0.35 ± 0.02 nmol/g wet tissue, P < 0.05; n = 5 for each group).

Basal levels of dopamine released into cortical dialysates of awake rats were similar in both groups (0.27 ± 0.17 and 0.29 ± 0.10 fmol/μl of dialysate for deficient and control rats, respectively). However, there were significantly higher metabolite levels in deficient rats than in controls (Fig. 1). The basal level of DOPAC was 74 ± 37 fmol/μl of dialysate in the deficient group versus 17 ± 10 fmol/μl in the control group. Similarly, the basal level of HVA was 64 ± 13 fmol/μl of dialysate in the deficient group versus 26 ± 11 fmol/μl in the control group (data are the mean ± SEM values of six samples for each rat without recovery correction; n = 8 for each group). KCl perfusion led to an increase in dialysate dopamine content for anaesthetized animals; the maximum response was 15 times higher than basal values for deficient and control rats (n = 8 for each group) (Fig. 2). This increase in dopamine level was accompanied by a reduction in the levels of DOPAC and HVA, without difference between groups (Fig. 3).

Tyramine perfusion resulted in a difference in dialysate dopamine levels between deficient and control rats. At the maximum increase, the basal dopamine levels were multiplied by 50 and 150 for the deficient and control group, respectively (n = 8 for each group) (Fig. 4). Dopamine-stimulated release was therefore significantly lower in deficient rats than in control rats (P < 0.05, Student t-test for unpaired values). In contrast, metabolite levels were not modified by the tyramine perfusion, and were similar in deficient and control rats (Fig. 5).

Previous studies in our laboratory have shown a lower endogenous dopamine level in the frontal cortex of α-linolenic acid-deficient rats compared to control rats [4,5]. Our current results agree with these earlier findings, showing...
that the endogenous dopamine level is lower in deficient animals. Dopaminergic function in the frontal cortex seems therefore to be affected by the fatty acid composition of the diet. This could be explained by several mechanisms, e.g. synthesis, storage or dopamine release, and therefore a dynamic approach to cortical dopaminergic neurotransmission was chosen for this study.

The results showed that in basal conditions DOPAC and HVA outputs were increased in deficient rats, without modification of the dopamine release. It is known that dopamine is metabolized in nerve endings by mitochondrial monoamine oxidase (MAO) to generate DOPAC, HVA being a secondary metabolite of DOPAC. Our results suggest that this metabolic pathway might be increased in α-linolenic acid-deficient rats. One likely explanation for this could be increased MAO activity. However, our previous study did not show any modification of this enzymatic activity in the deficient animal cortex [4]. The second possibility could be a modification of the metabolism of intraneuronal dopamine. In fact, under physiological conditions, most of the newly synthesized dopamine is stored in vesicles in the cytoplasm, being then protected from degradation by MAO. The unstored dopamine is largely catalyzed to DOPAC, which is then released, although part of this dopamine is released before metabolism. However, the mechanism triggering the conversion of non-releasable to releasable pools is unknown [8]. In our case, the unchanged basal dopamine level and the increased basal levels of DOPAC and HVA suggest a less efficient storage pathway that could result in reduction of the storage pool in deficient rats. In order to support this hypothesis, we studied both pools of intraneuronal dopamine, the cytoplasmic pool and the storage pool, with the microdialysis technique using specific in situ stimuli: a perfusion of KCl to induce release of newly synthesized dopamine, and a perfusion of tyramine to induce release of dopamine stored in the vesicular pool [6,9,19]. The modification of metabolites following the pharmacological stimulation lends further support to the origin of released dopamine: on the one hand, DOPAC and HVA levels were reduced by KCl, confirming that DOPAC is derived from the cytoplasmic dopamine [19]. On the other hand, dopamine metabolite levels were not diminished by tyramine. The KCl-induced release of dopamine was not different between deficient and control animals. In contrast, we demonstrated that the tyramine response was markedly reduced in deficient animals. It can therefore be hypothesized that n-3 PUFA deficiency has an effect on the dopamine vesicular pool in the frontal cortex. A reduction in synthesized dopamine could lead to decreased internalization in storage vesicles in deficient rats. However, the basal release of dopamine was identical in control and deficient awake rats, although the synthesis and release are not always co-varying. In addition, the endogenous level of cortical dopamine lends further support to the decrease in the constitution of the vesicular pool, the difference being caused by the lower levels of stored dopamine. Free dopamine might therefore be exposed to the mitochondrial MAO and be metabolized into DOPAC which is diffused into the synaptic cleft and yields HVA. The increase in DOPAC and HVA levels seems to support this interpretation. The mechanism of modification of storage processes in deficient rats remains unclear. Dopamine is stored in synaptic vesicles via the vesicular monoamine transporter (VMAT2). The vesicles in the reserve pool, in contrast, are linked to the cytoskeleton and are recruited to the release pool to maintain the physiological requirements of the cell. The deficit in n-3 PUFA could significantly alter the physical properties of vesicular membranes (fluidity, permeability and flexibility) as already suggested [10].

The decrease in dopamine available in the cortical vesicular pool can be juxtaposed with the modifications in behaviour already observed in deficient rats. The inadequate storage of newly synthesized dopamine might not be enough for the maintenance of high release during stimulated cognitive processes [8]. In addition, it has already been shown that learning situations may be accompanied by structural changes in the synapses of the cortex or hippocampus, and this may be affected by an alpha-linolenic acid-deficient diet [18]. In order to test our hypothesis, the study of cortical monoamine vesicles, and in particular their number and structure, is now in progress.

We thank Sylvie Bodard for her excellent technical assistance, and Doreen Raine for editorial assistance.


