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Docosahexaenoic acid, but not eicosapentaenoic acid, lowers ambulatory blood pressure and shortens interval QT in spontaneously hypertensive rats *in vivo*

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ABSTRACT

This study was designed to evaluate the effects of individual dietary long-chain n-3 polyunsaturated fatty acids (LCPUFA) on hypertension and cardiac consecutive disorders in spontaneously hypertensive rats (SHR) as compared to Wistar-Kyoto rats (WKY). Rats were fed for 2 months an eicosapentaenoic (EPA)- or docosahexaenoic acid (DHA)-rich diet (240 mg/day) or an n-3 PUFA-free diet. Male SHR ($n = 6$), implanted with cardiovascular telemetry devices, were housed in individual cages for continuous measurements of cardiovascular parameters (blood pressure (BP) and heart rate (HR)) during either activity or rest periods, ECG were recorded during the quiet period. The n-6 PUFA upstream of arachidonic acid was affected in SHR tissues. The cardiac phospholipid fatty acid profile was significantly affected by dietary DHA supply, and EPA in a very lower extent, since DHA only was incorporated in the membranes instead of n-6 PUFAs. Endothelium n-6 PUFA content increased in all SHR groups. Compared to WKY, linoleic acid content decreased in both studied tissues. Cardiac noradrenalin decreased while the adrenal catecholamine stores decreased in SHR as compared to WKY. Both n-3 PUFA supply induced a decrease of adrenal catecholamine stores. Nevertheless after 6 weeks, DHA but not EPA induced a lowering-blood pressure effect and shortened the QT interval in SHR, most probably through its tissue enrichment and a specific effect on adrenergic function. Dietary DHA supply retards blood pressure development and has cardioprotective effect. These findings, showing the cardioprotective effects of DHA in living animals, were obtained in SHR, but may relate to essential hypertension in humans.

1. Introduction

Approximately 90–95% of patients diagnosed with hypertension have primary (or essential) hypertension [1], whose physiopathology is a complex and polygenic age-related disorder resulting from the interaction of genetic and environmental factors, although no dominant mechanism has been identified as a main cause in humans [2] or in animals [3,4]. The changes in blood pressure (BP) and end-organ damage are life cycle disorders that are difficult to investigate, anticipate, and prevent in humans.

The spontaneously hypertensive rat (SHR) is widely used as an animal model for human essential hypertension and for the secondary consequences of hypertension *per se*. High BP is described in SHR as a consequence of peripheral vessel contraction and increased vascular resistance due to sympathetic stimulation hypersensitivity [4–6]. Sympathetic system overactivity, possibly associated with depressed parasympathetic nervous system activity, is largely considered to cause primary hypertension in humans and in SHR as well [6,7]. Moreover, left ventricle hypertrophy and fibrosis were shown to contribute to the progression of hypertension [8,9], parallel to particular and abnormal membrane fatty acid profile of hypertension target tissues in SHR [10]. Differences in FA composition may influence cell membrane structure and function, leading to perturbations in ion transport, receptor binding, and enzyme activities (for review, see [11,12]). As widely reported in the literature, the long-chain

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n-3 polyunsaturated FA (LCPUFA) displays pleiotropic effects that are still under investigation in n-3 PUFA, particularly regarding the prevention of cardiovascular disease (see for review [13–16]), involving effects on BP and lipid metabolism [17–19]. Experimental [20] and clinical studies [21] have shown an n-3 PUFA-induced decrease in BP in essential hypertension that is associated with altered n-3 PUFA content in plasma phospholipids [22]. Conversely, although some effects were reported in cases of moderate hypertension [17], fish oil BP-lowering effect remains controversial in established hypertension [23]. These discrepancies can be attributed to the large variability in experimental conditions, which includes intake level, population size, and experiment duration [24]. We previously reported that both dietary purified n-3 LCPUFA, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), reduced the rise of BP *in vivo* in both a renovascular hypertension model [18] and an insulin-resistant rat model [19,25]. EPA is mainly known to affect vasodilatation and platelet aggregation through its action on prostaglandins [26], whereas DHA has been reported to affect heart rate (HR) [19] and beta-adrenergic function [27,28]. However, in spite of a still renewed interest on n-3 PUFA cardioprotective potential [29], the individual and specific effect of EPA and DHA remains unknown, particularly in essential hypertension.

The present study was designed to investigate and discriminate the individual effect of n-3 LCPUFA, namely EPA and DHA, in the prevention of BP increase and the accompanying onset of cardiac impairments in the SHR model, parallel to the altered FA profile of endothelial cell and heart membrane.

2. Materials and methods

2.1. Physiopathological model and experimental diets

All animals received attentive care, and study protocols complied with the institutional guidelines for animal research (NIH Pub. no. 85-23, National Research Council, Revised 1996). The Animal Care and Use Committee of the Faculty of Pharmacy, University of Paris-Sud 11, approved the protocols. The animal holding facilities are registered under agreement number A 92-019-01. The main authors are authorized to manage experiments on animal (agreement level I, ref. 92-261, 2006-09-12). Eleven-week-old male SHR and age-matched normotensive Wistar-Kyoto rats (WKY) were purchased from CER Janvier (Le Genest-St-Isle, France). The WKY rat that is from the inbred rat strain, from which the SHR were derived, was employed as a control mostly for the biochemical dosages and tissue membrane FA profiles. The rats were housed in individual cages in a sound-attenuated room at constant humidity ($60 \pm 5\%$), temperature ($24 \pm 1\,^{\circ}C$), and a 12:12-h light–dark cycle throughout the experiment. The rats were first maintained on a standard rat diet (Safe A04, Epinay, France). After a 1-week acclimation period, 18 of the SHR were operated on as described later on, and after a 10-day surgical recovery period, all rats (SHR and WKY) were randomly assigned to three sub-groups based on their qualitative dietary fat intake. The rats were then maintained on different semi-purified jellied experimental diets and allowed access to tap water ad libitum. The experimental diets were prepared as previously described [18,19], with a formulation adapted from the recommendations of the AIN-93 [30]. Each rat was consuming 19–25 g of dry diet a day, without any significant difference between the groups. In the normal fat diet groups (NFD), the lipid fraction ($80\,g\,kg^{-1}$) was made of cocoa butter ($40\,g\,kg^{-1}$, Barry Callebaut, Meulan, France) and sunflower seed oil ($40\,g\,kg^{-1}$, Lesieur, France). In the n-3 PUFA-rich diets (DHA and EPA diets), the lipid part ($80\,g\,kg^{-1}$) was composed of cocoa butter ($40\,g\,kg^{-1}$), sunflower seed oil ($30.4\,g\,kg^{-1}$), and either DHA or EPA ($9.6\,g\,kg^{-1}$) supplied as ethyl esters (Hoffmann-LaRoche, Basel, Switzerland). Consumption of n-3 PUFA as ethyl ester or triglyceride has been shown to induce similar n-3 PUFA enrichments in serum lipids or platelets in humans [31,32]. The FA composition of the diets (Table 1) shows the characteristic trends of each diet, no n-3 PUFA in the NFD diet, rich in EPA in the EPA diet, or rich in DHA in the DHA diet; overall, these three diets have roughly similar contents in saturated (~30%, SFA), monounsaturated (~28%, MUFA), and PUFA (~40%).

Table 1
Dietary fatty acid profile (as % of total fatty acids) for NFD (without n-3 PUFA) or an EPA-rich and DHA-rich experimental diets.

Fatty acid	NFD	EPA	DHA
14:0	0.6	1.3	2.2
16:0	15.1	12.3	12.5
16:1 n-9	0.8	1.5	2.3
18:0	16.0	14.3	15.4
18:1 n-9	26.4	23.3	24.2
18:2 n-6	39.2	29.7	28.0
20:5 n-3	–	12.2	0.3
22:5 n-3	–	0.1	1.5
22:6 n-3	–	0.2	8.2
Minor SFA	0.6	1.4	2.2
Minor MUFA	0.9	2.6	2.3
Minor PUFA	0.5	1.6	1.2
Total SFA	32.3	29.3	32.3
Total MUFA	28.1	27.4	28.8
Total PUFA	39.7	43.8	39.2
Total n-6 PUFA	39.2	30.4	28.2
Total n-3 PUFA	0.5	13.3	10.8
n-6/n-3 ratio	84.1	2.3	2.6

NFD: normal fat diet group; EPA: 20:5n-3-enriched fat diet group; DHA: 22:6n-3-enriched fat diet group; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

2.2. Blood pressure and ECG measurements

To continuously monitor BP parameters, implanted telemetry transmitters (Data Science Inc., St. Paul, MI, USA) were surgically implanted only in 18 SHR, as previously described [19], 10 days prior to randomly assign these rats to the three experimental groups receiving one of the diets described above (NFD, DHA, and EPA groups) for an 8-week period. Cardiovascular parameters were then monitored in these telemeter-implanted rats between the ages of 14 and 22 weeks, a period characterized by the establishment of hypertension and progressive vascular and cardiac hypertrophies in SHR. The cardiovascular parameters were recorded under stress-free conditions for 10 s every 10 min, for each SHR rat 3 full days per week over the feeding period, as previously described [19]. We measured systolic BP (SBP), diastolic BP (DBP), pulse pressure (PP), and heart rate (derived from the pressure waveform) parallel to voluntary locomotors activity in freely moving unrestrained SHR. In addition to BP measurements, electrocardiogram (ECG) recordings were performed weekly during low activity periods on the SHR during the 8 weeks of dietary treatment, and body weight was recorded. The SBP of WKY rats were punctually measured by tail-cuff, as previously described [19].

2.3. Biochemical investigations

The SHR and WKY rats were killed under anaesthesia (pentobarbital intra-peritoneal injection, 50 mg/kg). Blood was