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Modulation of norepinephrine-stimulated cyclic AMP accumulation in rat pinealocytes by n-3 fatty acids

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Abstract

This work showed that docosahexaenoic (22:6n-3) and eicosapentaenoic (20:5n-3) acid supplementation for 48 h have opposite effects on the norepinephrine-stimulated cyclic AMP accumulation in rat pinealocytes. We found that 22:6n-3 supplementation of pineal cells, done by increasing specifically 22:6n-3 in phospholipid and triacylglycerol pools, led to inhibition of norepinephrine-stimulated cyclic AMP production whereas 20:5n-3 supplementation, by increasing 20:5n-3, 22:5n-3 and 22:6n-3 in the same pools, stimulated it. In contrast, direct treatment of pinealocytes with each fatty acid (50 μM) did not affect cyclic AMP production in the presence of (0.1–10 μM) norepinephrine. The results indicate that, using pharmacological agents such as forskolin or prazosin: (a) neither basal nor forskolin-stimulated cyclic AMP levels were modified in fatty acid-supplemented cells compared to control cells; (b) in the presence of 1 μM prazosin, the activation by 20:5n-3 was still effective whereas no additional inhibition of norepinephrine stimulation was observed in 22:6n-3-supplemented cells. Taken together, our results suggest that 22:6n-3 or 20:5n-3 supplementation modulates specifically the α_1- or β-adrenoceptors in the rat pineal gland.

Keywords: *n*–3 fatty acid; cAMP; Adrenoceptor; Norepinephrine; Pinealocyte; (Rat)

1. Introduction

The mammalian pineal gland is considered to be an endocrine organ, functioning almost exclusively for the synthesis and secretion of melatonin (Reiter, 1991). Norepinephrine is the primary physiological transmitter regulating pineal function. It stimulates pineal cyclic AMP and consequently melatonin synthesis via α_1- and β-adrenoceptors. β-Adrenoceptor activation stimulates adenylate cyclase directly via the regulatory protein Gs, and α_1-adrenoceptor activation, which itself has no apparent effect on cyclic AMP, potentiates β-adrenoceptor stimulation 10-fold (Vanecek et al., 1985). It has been reported that α_1-adrenoceptor activation increases both phospholipase C (Ho and Klein, 1987b; Ho et al., 1988a,b) and phospholipase A_2 activities (Ho and Klein, 1987a). The former enzyme generates diacylglycerol, which acts together with intracellular Ca^{2+} to activate a protein kinase C, which potently enhances β-adrenoceptor stimulation of cyclic AMP production (Sugden et al., 1985). The phospholipase A_2 enzyme is associated with release of arachidonic acid (20:4n-6) (Ho and Klein, 1987a), whose metabolic cascade has also been involved in the α_1-adrenoceptor potentiation of β-adrenoceptor-stimulated cyclic AMP production (Chik et al., 1991). We have reported that the rat pineal gland contains high proportions of polyunsaturated fatty acids, particularly linoleic acid (18:2n-6), 20:4n-6 and docosahexaenoic acid (22:6n-3) (Sarda et al., 1991). Moreover, the fatty acid composition of membrane pineal phospholipids is highly sensitive to n-3 fatty acid supplementation or deficiency (Sarda et al., 1991). Interestingly, results obtained with pineal from adult rats fed fish oil concentrates (enriched in 22:6n-3 and eicosapentaenoic acid, 20:5n-3) revealed a significant alteration of the fatty acid composition, especially a marked increase in 22:6n-3 and

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20:5n-3, associated with an increased synthesis of melatonin in response to norepinephrine stimulation (Sarda et al., 1992). Conversely, we have recently shown that dietary deprivation of n-3 fatty acids over three generations depleted the pineal content of 22:6n-3 and docosapentaenoic acid (22:5n-3) and induced a diminution of adenosine-stimulated (Gazzah et al., 1994) and norepinephrine-stimulated (Delton, 1995) cyclic AMP accumulation. From these in vivo observations, it became obvious that modifications in membrane lipid composition may result in functional changes in pineal activity, but the respective contribution of each n-3 fatty acid remained unknown.

To find whether enrichment of the membrane phospholipid and/or triacylglycerol pools was required to modify the physiological response in terms of norepinephrine-stimulated cyclic AMP accumulation in rat pinealocytes, we tested (a) the effect of a 5-min preincubation with 50 μM 22:6n-3 or 20:5n-3; (b) the effects of 48-h incubation with 25 and/or 50 μM of each fatty acid. Docosahexaenoic acid had an inhibitory effect whereas eicosapentaenoic acid exerted a stimulatory effect on norepinephrine-stimulated cyclic AMP production, both related with a specific enrichment of lipid pools. Use of pharmacological tools such as forskolin and prazosin yielded the preliminary results concerning their possible mechanisms of action on α_1- and β-adrenoceptors reported on here.

2. Materials and methods

2.1. Materials

Docosahexaenoic and eicosapentaenoic acids were obtained from Sigma (St Louis, MO, USA). Their purity was checked and their concentration was adjusted after gas-liquid chromatography measurement before each experiment. The docosahexaenoic acid preparation was relatively unstable and needed to be purified regularly. Pineal cell culture reagents (medium 199-Hepes, glutamine, gentamicin, hyaluronidase, DNase), free fatty acid bovine serum albumin, norepinephrine, prazosin, forskolin, and gas-liquid chromatography standards (diheptadecanoyl-glycerophosphocholine and of triheptadecanoyl-glycerol) were purchased from Sigma (St Louis, MO, USA). Collagenase was obtained from Boehringer-Mannheim (Meylan, France), and horse and fetal calf sera were from Flow Laboratories (Mc Lean, VA, USA). Silica gel 60 plates for thin-layer chromatography were from Merck (Darmstadt, Germany). The radioimmunoassay kit for cyclic AMP was purchased from the Pasteur Institute (Paris, France).

2.2. Rat pinealocyte preparation

All experiments were performed in accordance with French (87/848/Ministère de l'Agriculture et de la Forêt) and European Economic Community (86/609/EEC) guidelines for care and use of laboratory animals and were approved by a regional ethical committee for animal use.

Pineal glands were obtained from male Sprague-Dawley rats weighing around 200 g (Iffa Credo, France). They were housed at least one week prior to experimentation under conditions of controlled light and temperature, with food and water ad libitum. In all experiments, pineal glands were collected between 9:00 and 10:00 a.m. and immediately placed in medium 199-Hepes which was gassed with 95% air, 5% CO_2 at 37°C. Pineal glands were transferred into culture medium containing 1 mg/ml bovine serum albumin, 2 mM glutamine, 1 mg/ml collagenase, 0.5 mg/ml hyaluronidase and 10 μg/ml DNase, according to Delton (1995). The mixture was incubated for 30–40 min at 37°C in a humidified atmosphere (95% air, 5% CO_2) with manual shaking every 10 min to help cell dissociation. The reaction was stopped by adding an equal volume of fresh medium containing 2 mg/ml bovine serum albumin and the cell suspension was centrifuged at $500 \times g$ for 10 min. The cell pellet was resuspended in medium 199-Hepes containing 10% serum (mixture of 80% horse serum and 20% fetal calf serum), 2 mM glutamine and 50 μg/ml gentamicin, and incubated for 3 h prior to experiments.

2.3. Incubation with fatty acids

Fatty acids were saponified as previously described (Delton et al., 1995) and diluted to a final concentration of 50 μM in medium 199 containing 2.5% serum mixture 12 μM free fatty acid bovine serum albumin, 2 mM glutamine and 50 μg/ml gentamicin. The concentration of bovine serum albumin was 25 μM, taking into account the bovine serum albumin contained in serum, so that the fatty acid/albumin ratio was 2/1. The cells were centrifuged ($500 \times g$, 10 min) and resuspended either in control medium (without added fatty acid) or in supplemented medium (with added 22:6n-3 or 20:5n-3). Cell suspensions were then distributed as aliquots of 2 or 4×10^5 cells and incubated for 48 h in a humidified atmosphere. At the end of incubation, the cells were collected by centrifugation ($500 \times g$, 10 min). Aliquots of 4×10^5 cells were put into 500 μl water and kept at −20°C until fatty acid composition analyses. Aliquots of 2×10^5 cells were resuspended in 500 μl of the above control medium without serum and bovine serum albumin and maintained for 30 min under a humidified atmosphere before stimulation experiments.

2.4. Lipid analyses

Cellular lipids were extracted twice with chloroform/ethanol 2:1 (v/v). Butylated hydroxy toluene (50 μM) was added as an antioxidant and extractions were made in the presence of 5 μg each of diheptadecanoyl-glycerophosphocholine and triheptadecanoyl-glycerol as internal standards. Lipids were separated by thin-layer

chromatography on silica gel 60 plates using hexane/diethylether/acetic acid 70:30:1 (v/v) as developing solvent system. Total phospholipid and triacylglycerol pools were localized with reference to authentic standards, scraped off the plate, and collected into glass tubes. The fatty acid methyl esters were generated by heating at 100°C for 90 min with a mixture of boron trifluoromethanol and extracted with 2 ml isooctane and 1.5 ml K_2CO_3 5% (Morrisson and Smith, 1964). They were separated by gas-chromatography (Perkin-Elmer 8320) using a 0.32 mm by 60 m capillary column (high molecular weight polyethylene glycol) with helium as a carrier gas, identified by comparison with appropriate standards and quantified with internal standards.

2.5. Stimulation experiments and cyclic AMP assays

Each stimulation experiment was started by the addition of 50 μl norepinephrine or forskolin, a direct activator of adenylate cyclase, dissolved in water at adequate concentrations. Prazosin, an α_1-adrenoceptor antagonist, was preincubated 5 min before norepinephrine stimulation. After 15 min of treatment, cells were collected by centrifugation (2 min, $1000 \times g$), the supernatant was aspirated, the pellet was lysed by adding 200 μl perchloric acid 0.4 N and immediately frozen at −20°C. The cyclic AMP content was determined by RIA as previously described (Gharib et al., 1991).

2.6. Statistical analyses

The data are presented as the means ± S.E.M. Statistical analysis was carried out with Student's *t*-test.

3. Results

3.1. Dose-response curve for norepinephrine-stimulated cyclic AMP response in the presence of 22:6n-3 or 20:5n-3

Norepinephrine increased cyclic AMP in a concentration-dependent manner from 5.91 ± 0.52 pmol/200000 cells (basal level) to 11.98 ± 0.16 (0.1 μM), 21.55 ± 1.39 (1 μM) and 25.97 ± 1.47 pmol/200000 cells (10 μM). Preincubation for 5 min in the presence of 50 μM 22:6n-3 or 20:5n-3 did not cause any significant modification in this response. Under these conditions, each fatty acid remained unesterified, meaning that the lipid stores were not enriched (results not shown).

3.2. Effect of 22:6n-3 or 20:5n-3 supplementation on phospholipid and triacylglycerol fatty acid composition

In the following experiments, pinealocytes were incubated for 48 h with 25 or 50 μM 22:6n-3 or 50 μM 20:5n-3. This long-term fatty acid supplementation induced some fatty acid modifications in the two major lipid classes of rat pineal cells.

In phospholipids, supplementation by 25 μM 22:6n-3 induced a specific increase (+118%) in the 22:6n-3 level, from 0.55 to 1.20 nmol/4 × 10^5 cells. At 50 μM, 22:6n-3 supplementation induced an increase (+240%) of the 22:6n-3 level (from 0.55 ± 0.06 to 1.88 ± 0.21 nmol/4 × 10^5 cells) and of the level of 20:5n-3 (0.17 ± 0.03 nmol/4 × 10^5 cells) that was not detectable in control cells, indicating a slight retroconversion (Table 1). No other fatty acids were significantly affected by the 22:6n-3 supplementation. The supplementation with 50 μM 20:5n-3 induced an augmentation of the 20:5n-3 level in phospholipids, from not detectable to 0.68 ± 0.05 nmol/4 × 10^5 cells (Table 1). In addition, the levels of 22:5n-3 and 22:6n-3 were increased by 50% and 100%, respectively. Conversely, the levels of 18:1n-9, 18:2n-6 and 20:4n-6 were decreased by 60%, 100% and 80%, respectively.

In triacylglycerols, we found a specifically increased 22:6n-3 (8-fold compared to control cells) after 25 μM supplementation. As in the phospholipid pool, the supplementation with 50 μM 22:6n-3 resulted in a marked increase in the 22:6n-3 level, 12-fold compared to the control (from 0.42 ± 0.03 to 5.03 ± 0.56 nmol/4 × 10^5 cells) and in a slight retroconversion into 20:5n-3 (from not detectable to 0.11 ± 0.11 nmol/4 × 10^5 cells) (Table 2). We also observed a significant decrease of 18:1n-9 (−25%) and 18:1n-7 (−30%), that was too low (−0.97 nmol/4 × 10^5 cells) to compensate for the high increase in 22:6n-3 content (+4.61 nmol/4 × 10^5 cells). In consequence, the total amount of fatty acids in 22:6n-3 supple-

Table 1
Effects of 22:6n-3 or 20:5n-3 supplementation on the fatty acid composition of phospholipids

Fatty acids	Control (7)	22:6n-3 (8)		20:5n-3 (7)
		25 μM	50 μM	50 μM
18 DMA	0.26±0.02	0.27±0.04	0.31±0.04	0.23±0.10
16:0	2.05±0.16	2.18±0.27	1.91±0.25	1.57±0.16
18:0	3.93±0.30	3.55±0.27	4.27±0.32	3.37±0.35
18:1n-9	1.86±0.15	1.53±0.12	1.54±0.16	1.17±0.06 [a]
18:1n-7	0.18±0.01	0.16±0.02	0.18±0.01	0.14±0.02
18:2n-6	1.62±0.07	1.43±0.06	1.72±0.17	0.83±0.11 [c]
20:3n-6	0.12±0.02	0.13±0.01	0.16±0.02	0.12±0.04
20:4n-6	1.59±0.11	1.17±0.17	1.47±0.11	0.88±0.19 [b]
20:5n-3	ND	ND	0.17±0.03	0.68±0.05
22:4n-6	0.17±0.02	0.13±0.01	0.13±0.01	0.21±0.06
22:5n-3	0.15±0.01	0.12±0.01	0.13±0.01	0.23±0.03 [a]
22:6n-3	0.55±0.06	1.20±0.15 [c]	1.88±0.21 [c]	1.18±0.33 [c]
Total	12.82±0.90	11.91±0.99	13.94±0.90	10.93±0.89

Pinealocytes were maintained for 48 h in control medium or in medium supplemented with 25 or 50 μM 22:6n-3 or 50 μM 20:5n-3. The fatty acid composition of phospholipids was analyzed (see Materials and methods). Values are expressed as nmol/4×10^5 cells and represent means ± S.E.M. (*n*) the number of individual samples. ND: not detected. The total includes minor, not listed, unidentified fatty acids. Significance of differences was tested using Student's *t*-test: [a] $P \leq 0.05$, [b] $P \leq 0.01$, [c] $P \leq 0.001$ vs. control.

Table 2
Effects of 22:6n-3 or 20:5n-3 supplementation on the fatty acid composition of triacylglycerols

Fatty acids	Control (7)	22:6n-3 (10)		20:5n-3 (7)
		25 μM	50 μM	50 μM
16:0	3.43 ± 0.61	3.39 ± 0.62	2.81 ± 0.61	1.75 ± 0.23 [a]
18:0	2.16 ± 025	1.95 ± 0.13	2.06 ± 0.16	2.43 ± 0.24
18:1n-9	3.33 ± 0.25	2.79 ± 0.25	2.48 ± 0.24 [a]	1.83 ± 0.24 [c]
18:1n-7	0.34 ± 0.04	0.27 ± 0.04	0.22 ± 0.02 [a]	0.15 ± 0.01 [c]
18:2n-6	1.28 ± 0.05	1.05 ± 0.05	1.18 ± 0.07	0.99 ± 0.08 [b]
20:3n-6	0.15 ± 0.03	0.15 ± 0.02	0.20 ± 0.02	0.18 ± 0.03
20:4n-6	0.34 ± 0.03	0.42 ± 0.04	0.49 ± 0.05	0.79 ± 0.18 [a]
20:5n-3	ND	ND	0.11 ± 0.01	2.92 ± 0.50
22:4n-6	0.18 ± 0.03	0.17 ± 0.03	0.15 ± 0.02	0.13 ± 0.03
22:5n-3	0.16 ± 0.02	0.15 ± 0.01	0.16 ± 0.01	0.73 ± 0.22 [a]
22:6n-3	0.42 ± 0.03	3.45 ± 0.37 [c]	5.03 ± 0.56 [c]	1.78 ± 0.30 [c]
Total	12.29 ± 1.11	14.83 ± 1.24	15.53 ± 1.19	13.96 ± 0.25

Pinealocyes were maintained for 48 h in control medium or in medium supplemented with 25 or 50 μM 22:6n-3 or 50 μM 20:5n-3. The fatty acid composition of triacylglycerols was analyzed (see Materials and methods). Values are expressed as nmol/4 × 10^5 cells and represent means ± S.E.M. (n) the number of individual samples indicated. ND: not detected. The total includes minor, not listed, unidentified fatty acids. Significance of differences was tested using Student's t-test. [a] $P \leq 0.05$, [b] $P \leq 0.01$, [c] $P \leq 0.001$ vs. control.

mented cells tended to be greater (+25%) than in control cells. After supplementation with 50 μM 20:5n-3, the level of the latter became 2.92 ± 0.50 nmol/4 × 10^5 cells and those of 22:5n-3 and 22:6n-3 were increased by around 300% (Table 2). Conversely, decreased levels of 16:0 (−50%), 18:1n-9 (−50%), 18:1n-7 (−55%) and 18:2n-6 (−30%) were observed. Unexpectedly, a significant increase of 20:4n-6 occurred, that might compensate for the decrease of 18:2n-6 to maintain constant the total of the n-6 series fatty acids. The total increase in n-3 fatty acids (+5.3 nmol/4 × 10^5 cells) was partially compensated for by the total decrease in n-6 fatty acids (−3.66 nmol/4 × 10^5 cells) (Table 2).

3.3. Effect of the fatty acid modification on cyclic AMP response to norepinephrine stimulation

Fatty acid supplementation with either 22:6n-3 or 20:5n-3 had no effect on basal cyclic AMP levels. The cyclic AMP response to norepinephrine (1 and 10 μM) was decreased (around −20%) in the 50 μM 22:6n-3 supplemented cells: 17.36 ± 0.96 vs. 21.15 ± 1.09 pmol/2 × 10^5 cells and 20.05 ± 1.77 vs. 25.40 ± 1.29 pmol/2 × 10^5 cells, respectively (Fig. 1). The pattern was similar with 25 μM 22:6n-3 (data not shown). In contrast, in 50 μM 20:5n-3 supplemented cells cyclic AMP accumulation was increased after norepinephrine stimulation in a concentration-dependent manner (from +20 to +40%). The respective cyclic AMP levels in control and 20:5n-3 supplemented cells were 11.02 ± 0.20 and 15.47 ± 0.33 pmol/2 × 10^5 cells at 0.1 μM norepinephrine, 21.15 ± 1.09 and 27.74 ± 0.35 pmol/2 × 10^5 cells at 1 μM, and 25.40 ± 1.29 and 30.26 ± 0.91 pmol/2 × 10^5 cells at 10 μM (Fig. 2).

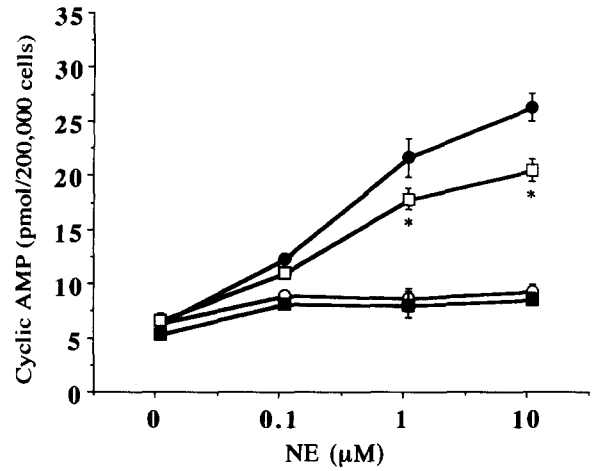

Fig. 1. Effects of 22:6n-3 supplementation on norepinephrine-induced cyclic AMP accumulation in rat pineal cells. Pinealocytes were incubated in control medium or in medium supplemented with 50 μM 22:6n-3 for 48 h as described in Materials and methods. Cells of each group: control cells (●); 50 μM 22:6n-3 supplemented cells (□); 1 μM prazosin cells (○); 1 μM prazosin + 50 μM 22:6n-3 supplemented cells (■) were then resuspended in control medium and treated with norepinephrine (0.1–10 μM). The cyclic AMP content was determined by RIA 15 min after norepinephrine stimulation. Values are expressed as pmol/200000 cells and represent means ± S.E.M. from five individual samples. * Different from control at $P \leq 0.05$.

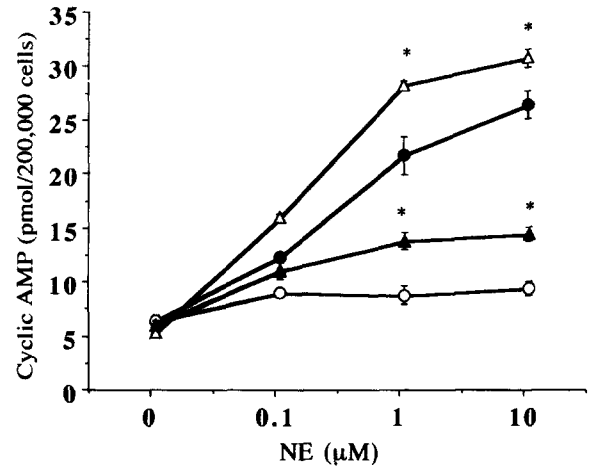

Fig. 2. Effects of 20:5n-3 supplementation on norepinephrine-induced cyclic AMP accumulation in rat pineal cells. Pinealocytes were incubated in control medium or in medium supplemented with 50 μM 20:5n-3 for 48 h as described in Materials and methods. Cells of each group: control cells (●); 50 μM 20:5n-3 supplemented cells (△); 1 μM prazosin cells (○); 1 μM prazosin + 50 μM 20:5n-3 supplemented cells (▲) were then resuspended in control medium and treated with norepinephrine (0.1–10 μM).The cyclic AMP content was determined by RIA 15 min after norepinephrine stimulation. Values are expressed as pmol/200000 cells and represent means ± S.E.M. from five individual samples. * Different from control at $P \leq 0.05$.

Table 3
Effect of 50 μM 22:6n-3 or 20:5n-3 supplementation on forskolin-stimulated cyclic AMP responses in rat pineal cells

Treatment		Control	22:6n-3	20:5n-3
Basal		2.69 ± 0.40	2.03 ± 0.24	2.05 ± 0.17
Forskolin	1 μM	12.61 ± 0.83	11.45 ± 0.77	10.55 ± 0.74
	10 μM	15.90 ± 1.57	13.34 ± 1.00	16.99 ± 0.52
	100 μM	19.57 ± 0.26	20.12 ± 1.97	18.67 ± 0.71

Pinealocytes were incubated in control medium or in medium supplemented with 50 μM 22:6n-3 or 20:5n-3 for 48 h. Cells were then resuspended in control medium and stimulated with forskolin at different doses. The cyclic AMP content was determined by RIA 15 min after stimulation. Values are expressed as pmol/2 × 10^5 cells and represent means ± S.E.M. from five individual samples.

3.4. Effect of fatty acid supplementation on forskolin stimulation

In control cells, treatment with forskolin (1, 10 and 100 μM) increased cyclic AMP accumulation in a concentration-dependent manner (Table 3). Neither 22:6n-3 nor 20:5n-3 supplementation modified cyclic AMP accumulation significantly (Table 3).

3.5. Involvement of α_1- and β-adrenoceptor systems

The α_1-adrenoceptor antagonist prazosin (1 μM), used to block the weak α_1-adrenoceptor effect of norepinephrine, did not affect the basal cyclic AMP level but decreased the norepinephrine-stimulated cyclic AMP responses (up to −60% at 10 μM norepinephrine) in the control group. In this case, the cyclic AMP levels reflected only stimulation of β-adrenoceptors. 22:6n-3 supplementation did not alter the response to norepinephrine-prazosin co-treatment (Fig. 1). In contrast, in 20:5n-3 supplemented cells, increases in cyclic AMP accumulation were more pronounced by 25 to 60% (10.55 ± 0.27 vs. 8.42 ± 0.35 pmol/2 × 10^5 cells at 0.1 μM, 13.23 ± 0.79 vs. 8.26 ± 0.85 pmol/2 × 10^5 cells at 1 μM, and 13.91 ± 0.71 vs. 9.86 ± 0.68 pmol/2 × 10^5 cells at 10 μM) (Fig. 2).

4. Discussion

The primary aim of this study was to define the respective contribution of 22:6n-3 and 20:5n-3 to the modulation of the pineal activity.

In vivo dietary experiments dealing with n-3 fatty acids are generally characterized by compensation between the n-3 and n-6 series fatty acids. This phenomenon has been observed in rat pineal glands after both n-3 supplementation (Sarda et al., 1991) and n-3 deficiency (Gazzah et al., 1994). In the present study, we found that in vitro supplementation with 25 μM 22:6n-3 for 48 h increased specifically the 22:6n-3 level in the phospholipid and triacylglycerol pools, with a small increase of 20:5n-3 when 50 μM 22:6n-3 was used. In contrast, supplementation with 50 μM 20:5n-3 increased the level of 20:5n-3, 22:5n-3 and 22:6n-3, together reducing reciprocally the level of some n-9 and n-6 series fatty acids. These results show that the supplementation by 20:5n-3 reproduces approximately the changes induced by fish oil concentrates in vivo, in particular with a decrease in 18:2n-6 and 20:4n-6 contents to compensate for the increase in n-3 fatty acids (Sarda et al., 1991). After 20:5n-3 supplementation in vitro, the total amount of fatty acids remained unchanged both in the phospholipid and triacylglyceol pools, indicating exchanges via the deacylation/acylation mechanism. This mechanism did not seem to operate during supplementation by 22:6n-3, especially in triacylglycerols whose mass tended to be enhanced, as also described for hepatocytes (Martin et al., 1991). This suggests that the increase in 22:6n-3 content in this lipid class was due to de novo synthesis rather than to deacylation/acylation. That both 22:5n-3 and 22:6n-3 levels were enhanced after 20:5n-3 supplementation indicates that pineal cells were able to elongate and desaturate 20:5n-3. In contrast, the retroconversion of 22:6n-3 into 20:5n-3 was very low. Taken together, these results show that the metabolism of n-3 fatty acids in rat pinealocytes is essentially oriented towards the synthesis of 22:6n-3, in agreement with their endogenous fatty acid composition (Sarda et al., 1991; Sawazaki et al., 1994). The elongation/desaturation of 20:5n-3 into 22:6n-3 has also been described for retinal pigment epithelium (Wang and Anderson, 1993), astrocytes (Moore et al., 1991) and astroglia (Tocher, 1993).

Our present study confirmed the influence of n-3 fatty acids on pineal activity in terms of cyclic AMP production, and illustrated the effects of 22:6n-3 and of 20:5n-3. Indeed, 22:6n-3 supplementation, by increasing 22:6n-3, reduced the norepinephrine-stimulated cyclic AMP response, whereas 20:5n-3 supplementation by increasing 20:5n-3, 22:5n-3 and 22:6n-3, increased this response. Tesoriere et al. (1988) reported that, in bovine retinal cells, 22:6n-3 treatment in the concentration range of 0.5 μM to 1 mM enhanced epinephrine-stimulated adenylyl cyclase activity. In contrast, short-term incubation with 50 μM 22:6n-3 or 20:5n-3 did not affect the cyclic AMP production after norepinephrine stimulation. That 22:6n-3 and 20:5n-3 did not affect basal or forskolin-stimulated cyclic AMP levels, but changed cyclic AMP accumulation after norepinephrine stimulation, might indicate that the fatty acid effect did not occur directly on adenylyl cyclase. In pineal gland, the inhibitory effect of 22:6n-3 supplementation was unexpected with regard to previous in vivo studies that had shown a positive relationship between the n-3 fatty acid content and the cyclic AMP responses (Sarda et al., 1992; Gazzah et al., 1994). This apparent discrepancy may suggest that 22:6n-3 was incorporated in distinct compartments of the cellular lipid pools depending on whether it came from exogenous or endogenous sources.

It is well known that norepinephrine stimulation induces

cyclic AMP accumulation in rat pinealocytes by involving a synergistic mechanism between α_1- and β-adrenoreceptors. As previously described (Vanecek et al., 1985), the treatment with the α_1-adrenoceptor antagonist, prazosin, provoked a 25 to 60% decrease of the norepinephrine-induced cyclic AMP responses in control cells. As no additional inhibition was observed in 22:6n-3-supplemented pinealocytes, we may hypothesize that 22:6n-3 acts on α_1-adrenoceptor sites. In contrast, the activator effect of norepinephrine in 20:5n-3-supplemented cells was effective in the presence of prazosin, suggesting that the 20:5n-3 effect could be independent of α_1-adrenoceptor stimulation. Assuming that, in the presence of prazosin, the cyclic AMP response was due only to the β-adrenoceptor stimulation, we may speculate that 20:5n-3 supplementation enhanced the β-norepinephrine response by affecting the β-adrenoceptor sites. Consistent with this a previous study had shown that dietary 20:5n-3 increased the Kd value of cardiac β-adrenoceptors in the marmoset monkey (Patten et al., 1989).

Finally, evidence for the incorporation of 20:5n-3 and 22:6n-3 into membranes to modulate fluidity, has been reported for nerve membranes (Bourre et al., 1993). It may then be assumed that cyclic AMP production could be controlled by means of local variation in membrane fluidity, which in turn could affect the coupling between the receptors (α_1/β) and adenylyl cyclase activity. Further experiments are in progress, including binding experiments, to ascertain the specific mechanism of the 20:5n-3 or 22:6n-3 effect on the α_1- or β-adrenoceptor/transducing system in the rat pineal gland.

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