Long-Chain Polyunsaturated Fatty Acids Influence Both β- and α-Adrenergic Function of Rat Cardiomyocytes

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ABSTRACT: Dietary polyunsaturated fatty acids (PUFA) have been reported to lower the incidence of cardiovascular diseases, but neither the mechanisms that determine these protective effects nor the specific influence of n-3 vs. n-6 PUFA series has been well established. The purpose of this work was to demonstrate the influence of the membrane long-chain fatty acid composition on the spontaneous contractile activity and the adrenergic function of rat cardiomyocytes in culture. Cells were grown for 24 h in a standard culture medium and then for 4 d in media that contained either n-3 (eicosapentaenoic acid and docosahexaenoic acid) or n-6 (arachidonic acid) PUFA. The n-6/n-3 ratio was 1.2 in n-3 cells compared with 20.1 in n-6 cells. The basal contractile properties of these cardiomyocytes were not affected by the PUFA phospholipid composition. However, these modifications influenced the adrenoceptor function because the β-adrenergic stimulation by isoproterenol (10^{-7} M) induced a positive chronotropic response that was significantly greater in n-3 cells. Moreover, the chronotropic response to α-adrenoceptor stimulation by phenylephrine (10^{-6} M) appeared significantly more pronounced in the n-3 cells than in the n-6 cells. However, the parameters related to the inotropic response induced by these agonists did not differ significantly between the two groups of cells. These results suggested that the membrane long-chain PUFA composition does not influence the basal cardiac contractility and automaticity but is able to modulate the chronotropic function of both α- and β-adrenoceptors.

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KEY WORDS: α- and β-Adrenoceptors, arachidonic acid, automaticity, cardiomyocytes, contractility, docosahexaenoic acid, eicosapentaenoic acid.

Epidemiological as well as primary and secondary prevention studies of ischemic heart diseases have revealed the protective effects of diets that are enriched with n-6 or n-3 polyunsaturated fatty acids (PUFA) (1–4). In particular, evidence from Greenland Eskimos and Japanese populations has suggested that marine oils, rich in n-3 PUFA, have a beneficial effect on many cardiovascular risk factors (1). In humans, these effects have often been described in terms of their hypolipemiant (2), hypotensive (5) and antiaggregant actions (6). Nevertheless, the recent work of Lervang *et al.* (3) brings into question the influence of n-3 PUFA on the strictly vascular parameters and proposes that their beneficial effects may be mediated through other mechanisms.

In this context, a direct influence of PUFA on the myocardial function has been suggested. Through changes in the degree of unsaturation of diet fatty acids, modifications in the cardiac lipid composition were obtained (4,7). Under physiological conditions, such changes influence the contraction rate and the systolic ejection volume of the rat heart (8). In addition, the activities of some functional proteins associated with the membrane are assumed to be modified. In particular, Charnock *et al.* (9) showed that an increase of the phospholipid (PL) n-6/n-3 PUFA ratio is accompanied by a decrease in the Ca^{2+} channel density, whereas this alteration does not affect the adenylate cyclase activity in the monkey (10).

Little consideration has been given in turn to the specific effects of PUFA on the myocardial reactivity to autonomic neurotransmitters. Studies on the effects of dietary fatty acids on adrenergic receptor binding characteristics in the rat led to controversial data. Fish oils were reported to affect the vascular and cardiac noradrenaline sensitivity through alterations in receptor affinity and density (11–14). Likewise, it was established that n-6 PUFA provoke a decrease in the β-adrenoceptor density and an increase in their affinity (15). The influence of diet on the functional response of adrenoceptors was also studied, but differences in methodological approaches led to conflicting data. Reibel *et al.* (16) showed in perfused heart that fish oils alter the inotropic response to α-adrenergic stimulation but not the response to a β-agonist. Earlier *in vitro* investigations with cultured cardiomyocytes (CM) demonstrated that the n-6/n-3 ratio of PUFA influences the β-adrenergic response, whereas the α-response is unaffected (17).

There is thus a need to investigate more directly the influence of the n-6 long-chain PUFA, compared to the n-3 series, at the myocardial cell level. However, modifications in fatty acid supply can affect every cell type present in the cardiac tissue, such as CM, endothelial cells, and fibroblasts. Analysis of the fatty acid influence on the cardiac muscular function should be approached through the use of isolated myocardial cell preparations. With ventricular cell cultures, we

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have shown previously that the PL PUFA composition of CM can be modified through a change in culture medium (18) and that these modifications influence the β-adrenergic function only (17). However, these preliminary studies with precursor fatty acids (C18) led to minor changes in the membrane long-chain PUFA composition, the ventricular myocytes being unable to desaturate the n-3 fatty acids. Therefore, the purpose of the present work is to reassess the respective influence of n-6 and n-3 PUFA on the adrenergic function of isolated CM through direct supplementation of the culture medium in long-chain PUFA. In this aim, cultivated rat ventricular cells were incubated in media enriched either in arachidonic acid (AA) or in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Then, the effects of changes in cellular PL composition upon the basal contractile properties of the CM and on their responses to α- and β-adrenergic stimulation were evaluated.

MATERIALS AND METHODS

Media and solutions. The standard culture medium (HamC Med) was composed of Ham's F10 medium (Seromed, Berlin, Germany), supplemented with 10% fetal calf serum (Seromed), 10% human serum, and antibiotics. Two fatty acid-enriched media were prepared from HamC Med, an n-6 rich medium (n-6 Med) supplemented with AA (C20:4n-6), and an n-3 medium (n-3 Med) supplemented with EPA (C20:5n-3) and DHA (C22:6n-3). The final fatty acid compositions of these two media are presented in Table 1. In all media, pH was adjusted at 7.4. Sterilization was achieved by ultrafiltration through sterile 0.22 µm filters (Millipore, Bedford, MA). The calcium activity of all media was standardized at 1.2 mM just before use by addition of a sterile solution of $CaCl_2 \cdot 2H_2O$ (27 mg/mL; Merck, Darmstadt, Germany).

Cell culture preparation. Primary cultures of rat ventricular myocytes were prepared as previously described (19). The hearts were removed aseptically from 2- to 4-day-old Wistar rats. The ventricles were minced, and the cardiac cells were dissociated during seven consecutive proteolytic treatments in 0.1% trypsin (Difco, Detroit, MI) at 30°C. The cells from the last six steps were resuspended in HamC Med. Myocyte enrichment was performed by a two-step differential attachment procedure. The cell suspension was seeded in 60-mm Petri dishes at a density of $2 \cdot 10^6$ cells/dish and incubated at 37°C in a humidified atmosphere that contained 5% CO_2, 19% O_2, and 76% N_2. After 24 h in HamC Med, the cells were grown in either HamC Med or one of the two fatty acid-enriched media for four additional days, and then they were submitted to the experimental processings.

TABLE 1
Fatty Acid Composition[a] of the Standard Culture Medium (HamC Med) and the Experimental Media n-6 Med and n-3 Med

Fatty acid	HamC Med	n-6 Med	n-3 Med
16:0	22.6	20.6	20.1
18:0	8.1	7.3	7.3
18:1	20.9	18.9	18.6
18:2n-6	27.8	27.9	27.7
20:4n-6	6.7	12.5	6.1
20:5n-3	0.6	0.6	4.6
22:6n-3	2.0	1.7	6.5

[a]Values expressed as percentage of total fatty acids ($n = 3 \times 3$: three sets of each medium); minor fatty acids were omitted.

Fatty acid analysis. The media were freeze-dried, and the fatty acids were transmethylated without previous extraction (20). In the cell, the lipids were extracted according to Folch *et al.* (21), and the PL were separated from the nonphosphorus lipids in silica cartridges (22). Then, the PL fatty acids were analyzed by gas chromatography on a Carbowax 20M capillary column after transmethylation with BF_3-methanol.

Physiology. The experiments were performed in Puck's F balanced salt solution under static bath conditions. This experimental medium was covered with a paraffin oil layer. The culture dish was fixed in a heated circulating water chamber (23,24) on the moving stage of an inverted phase-contrast microscope (Leitz Diavert, Wetzlar, Germany). The dish was maintained at 36°C under constant air flux (700 mL/min). The main values of pH, PO_2 and PCO_2 were 7.36 ± 0.02, 96 ± 5.33, and 20.7 ± 0.84 mm Hg, respectively, and were stable during the complete experiment.

An image of the beating CM was produced by a video camera. The cell contractions were monitored on a TV monitor screen by a photoelectric transducer as previously described (17). The contraction signals were displayed by a storage oscilloscope (Gould DSO 1604, Longjumeau, France) and transcribed on a paper chart with an ink-jet recorder (Siemens EM 81, Solna, Sweden). The analysis of acquired data and calculations were performed on a PC/AT personal computer with an ASYST-based processing program (Keithley Instrument, Taunton, MA).

Pharmacology. Stock solutions of isoproterenol and phenylephrine were prepared in Puck's F salt solution and kept at 0–4°C in the dark until use. The cells were treated by addition of 10 µL of each of these solutions with a Hamilton syringe (Bonadag, Switzerland) driven by a micromanipulator. The final concentrations of isoproterenol and phenylephrine (PHE) in the culture dishes were 10^{-7} and 10^{-6} M, respectively.

Statistics. For basal contractility, six experiments for each group of cells were performed in cultures from different preparations. In each experiment, the measurements of the physiological parameters were carried out in five areas of the dish. The data were submitted to a two-way analysis of variance (25) with a fixed factor (medium) and a random factor (dish).

In the pharmacological studies, seven dishes for each group of cells were used in the isoproterenol experiments and six dishes in the PHE experiments. Five measurements were carried out in each dish during the pretreatment period and then five measurements 10 min after addition of the drug. Comparison of the effects of isoproterenol and phenylephrine between n-3 and n-6 cells was achieved by a three-way analysis of variance on data expressed as percentages of change induced by the drug.

RESULTS

PL PUFA. The fatty acid composition of cell PL is shown in Figure 1A. In the three groups of cells, the contents in saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and PUFA were similar. The major differences were found in the PUFA fraction because the PL n-6/n-3 ratio was 20.1 in the n-6 cells and 1.2 in the n-3 cells. The PL of the n-3 cells were characterized by a high content in both EPA (5.5%) and DPA (docosapentaenoic acid, C22:5n-3; 4.3%) and in DHA (8.3%) (Fig. 1B). Those of the n-6 cells appeared enriched in AA (28.1 vs. 12.5% for n-3 cells) and in docosatetraenoic acid (C22:4n-6; 8.2%).

Contractile properties. The contractile parameters of cells grown in standard medium (HamC cells) are presented in Table 2. The contraction rate (CR) was in the range of 181.7 to 313.6 cycles/min, with a mean of 230.1 cycles/min. The mean values of contraction duration at 20 (CD20) and 80% relaxation (CD80) were 77.7 and 188.4 ms, respectively. The time for 20 to 80% contraction ($+C_{max}$), which expresses the shortening time, reached 49.3 ms and varied from 38 to 66 ms. The relaxation time ($-C_{max}$) ranged between 46 and 78 ms, with an average value of 63.4 ms.

The mean values of the contraction parameters in each experimental group are summarized in Table 2. The fatty acid composition did not affect the contractile properties of CM under basal conditions. The apparent increase in CR of the n-6 cells was not significant. Moreover, modifications of the n-6/n-3 PUFA ratio had no influence on the contraction duration at 20 (CD20) and 80% (CD80) relaxation (76.8 and 185 ms, respectively). These modifications also did not influence the shortening time ($+C_{max}$) and relaxation time ($-C_{max}$) (49.5 and 61.6 ms, respectively).

Effect of isoproterenol. The amplitude and time course of the chronotropic effect induced by isoproterenol are shown in Figure 2. In response to the addition of isoproterenol (10^{-7} M) to the bath, a gradual increase in spontaneous beating rate appeared within 2 min. The values of measured contraction parameters are listed in Table 3. In the two experimental groups, the β-agonist caused an increase in spontaneous beating rate. This positive chronotropic effect was accompanied by a shortening of the contraction duration indexes (CD20 and CD80). Additionally, a decrease in the times for 20 to 80% contraction and relaxation ($+C_{max}$ and $-C_{max}$, respectively)

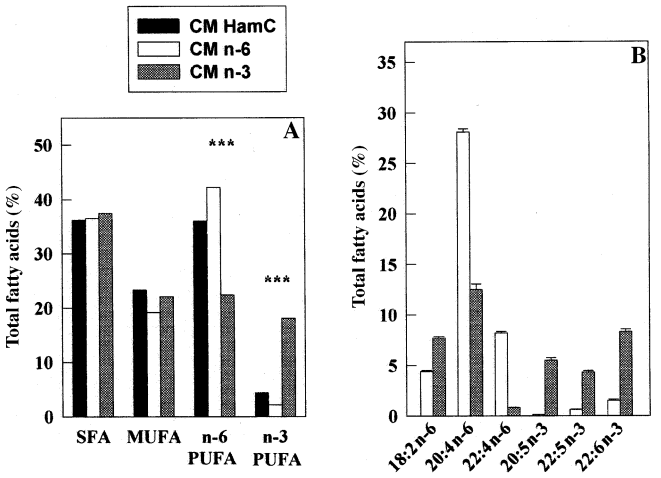

FIG. 1. (A) Total fatty acid composition of membrane phospholipids of rat cardiomyocytes (CM), incubated 4 d in the different incubation media ($n = 3 \times 3$: three dishes in each group of CM). Media are described in the Materials and Methods section. (B) PUFA phospholipid composition of rat cardiomyocytes, incubated 4 d in n-6 and n-3 media. Data are expressed as percentage of total fatty acids. Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CM, cardiomyocytes. $^{***}P < 0.01$. Bars indicate standard deviation of the mean.

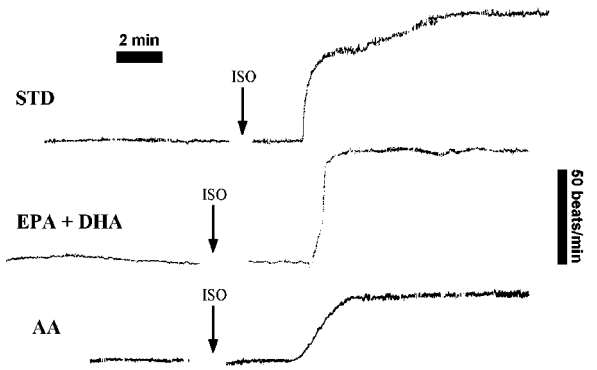

FIG. 2. Isoproterenol (10^{-7} M) effect on the contraction rate of rat ventricular myocytes, incubated 4 d in the three different media. Abbreviations: STD, HamC cells; EPA + DHA, n-3 cells; AA, n-6 cells; ISO, isoproterenol; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid.

TABLE 2
Basal Contractile Parameters of Cardiomyocytes, Incubated for 4 d, Either in HamC Med or in the Fatty Acid-Controlled Media n-3 Med and n-6 Med[a]

	CR (cycles/min)	CD20 (ms)	CD80 (ms)	$+C_{max}$ (ms)	$-C_{max}$ (ms)
CM HamC	230.1 ± 14.8	77.7 ± 7.8	188.4 ± 11.7	49.3 ± 2.2	63.4 ± 3.7
CM n-6	250.4 ± 23.8	76.8 ± 9.3	185 ± 17.3	49.5 ± 3.2	61.6 ± 4.9
CM n-3	225.5 ± 20.3	81.6 ± 9.3	191.2 ± 16.2	51 ± 3.0	62.5 ± 4.7
ANOVA	NS	NS	NS	NS	NS

[a]Means ± SEM ($n = 13$). Abbreviations: CR, contraction rate; CD20, contraction duration at 20% relaxation; CD80, contraction duration at 80% relaxation; $+C_{max}$, time for 20 to 80% shortening; $-C_{max}$, time for 20 to 80% relaxation; CM, cardiomyocytes; NS, not significant.

TABLE 3
Effect of Isoproterenol (ISO) on Contractile Parameters of Cultured Cardiomyocytes, Incubated for 4 d in the Fatty Acid-Controlled Media n-3 Med and n-6 Med[a]

	CR (cycles/min)	CD20 (ms)	CD80 (ms)	$+C_{max}$ (ms)	$-C_{max}$ (ms)
CM n-6					
Basal	299.4 ± 26.2	58.4 ± 5.6	150.6 ± 11.9	43.0 ± 3.2	50.4 ± 3.5
ISO	340.9 ± 24.2	48.5 ± 3.9	127.5 ± 8.9	37.1 ± 2.7	43.7 ± 3.2
ANOVA	<0.01	<0.05	<0.01	<0.05	<0.01
CM n-3					
Basal	261.3 ± 13.9	63.2 ± 4.0	161.7 ± 8.8	45.7 ± 1.8	54.2 ± 2.5
ISO	329.3 ± 23.2	51.2 ± 3.6	132.1 ± 9.7	37.4 ± 2.3	43.5 ± 3.0
ANOVA	<0.01	<0.001	<0.001	<0.05	<0.01

[a]Means ± SEM (n = 7). For abbreviations see Table 2. ANOVA (analysis of variance) relates to the efficiency of the adrenergic agonist effect. For comparison of CM n-3 and CM n-6, refer to Figure 3.

was observed. Because the shortening and relaxation velocities, measured from isolated myocardial cells, were used as an index of contractility, the decrease in shortening and relaxation time observed here can be considered as a direct isoproterenol-induced inotropic response. The differences in the PUFA composition of the membrane PL influenced the chronotropic effect induced by isoproterenol. Comparison of the chronotropic responses obtained (Fig. 3) showed that the increase in beating rate was significantly greater in the n-3 cells than in the n-6 cells because the addition of isoproterenol increased the CR by 25% in the n-3 cells and by 15% in the n-6 cells. The isoproterenol-induced modifications of the other contraction parameters were not significantly influenced by the n-6/n-3 ratio of the cell PL, although the largest decreases in the contraction duration indexes and in the shortening and relengthening times were observed in the n-3 cells.

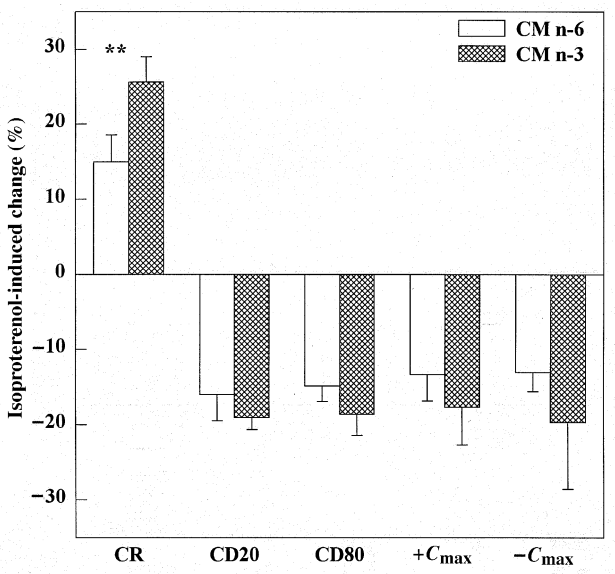

FIG. 3. Relation between phospholipid PUFA composition and isoproterenol effects on the contractile parameters. Data are expressed as percentage of change induced by isoproterenol stimulation. Values are means ± SEM (n = 7). $^{**}P < 0.05$. Abbreviations: CR, contraction rate; CD20, contraction duration at 20% relaxation; CD80, contraction duration at 80% relaxation; $+C_{max}$, time for 20 to 80% shortening; $-C_{max}$, time for 20 to 80% relaxation. For other abbreviations see Figure 1.

Effect of PHE. The effects of PHE addition (10^{-6} M) on myocardial cell automaticity and contractility are shown in Figure 4. PHE induced a gradual increase of the spontaneous beating rate within 2 min. The peak response was followed by a slight decline to a steady level, during which measurements were made. The corresponding changes in contraction parameters are presented in Table 4. Whatever the incubating medium, addition of the α-agonist significantly increased the beating rate. This positive chronotropic response was accompanied by a decrease in the contraction duration (CD20 and CD80). However, the n-3 and n-6 cells seemed to differ in this regard because the PHE-induced contraction shortening was significantly more pronounced in the n-3 cells. A significant decrease in the shortening time ($+C_{max}$) was observed in the two groups of cells. Conversely, the addition of PHE decreased significantly the relaxation time ($-C_{max}$) only in n-3 cells.

A diagrammatic comparison of the chronotropic responses to PHE between the two cell groups is presented in Figure 5. These responses differed with respect to the PUFA composition of the PL because the increase in rate was significantly greater in the n-3 cells than in the n-6 cells (+28 and +13%, respectively). In contrast, the other contractile characteristics were not significantly different between the n-6 and the n-3 cells. However, all these parameters tended to decrease to a greater extent in n-3 cells.

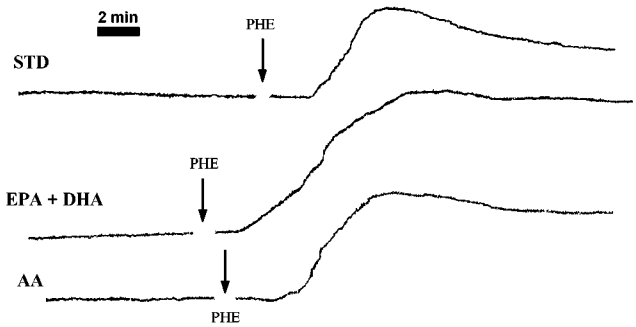

FIG. 4. Phenylephrine (PHE) (10^{-6} M) effects on the contraction rate of rat ventricular myocytes, incubated 4 d in the three different media. For other abbreviations see Figure 2.

TABLE 4
Effect of Phenylephrine (PHE) on Contractile Parameters of Cardiomyocytes, Incubated for 4 d in the Fatty Acid-Controlled Media n-3 Med and n-6 Med[a]

	CR (cycles/min)	CD20 (ms)	CD80 (ms)	$+C_{max}$ (ms)	$-C_{max}$ (ms)
CM n-6					
Basal	193.3 ± 23.43	98.2 ± 14.09	225.1 ± 25.48	57.1 ± 3.78	74.6 ± 5.92
PHE	217.6 ± 24.39	78.8 ± 10.41	189.9 ± 24.37	51.5 ± 4.78	66.6 ± 8.02
ANOVA	<0.01	NS	NS	<0.05	NS
CM n-3					
Basal	183.8 ± 32.66	102.9 ± 14.81	225.6 ± 26.7	57.3 ± 4.98	72.1 ± 7.94
PHE	229.9 ± 37.33	76.7 ± 9.65	186.2 ± 19.97	48.6 ± 4.17	64.3 ± 6.22
ANOVA	<0.01	<0.05	<0.01	<0.05	<0.05

[a]Means ± SEM (n = 6). For abbreviations see Tables 2 and 3. The ANOVA relates to the efficiency of the adrenergic agonist effect. For comparison of CM n-3 and CM n-6, refer to Figure 5.

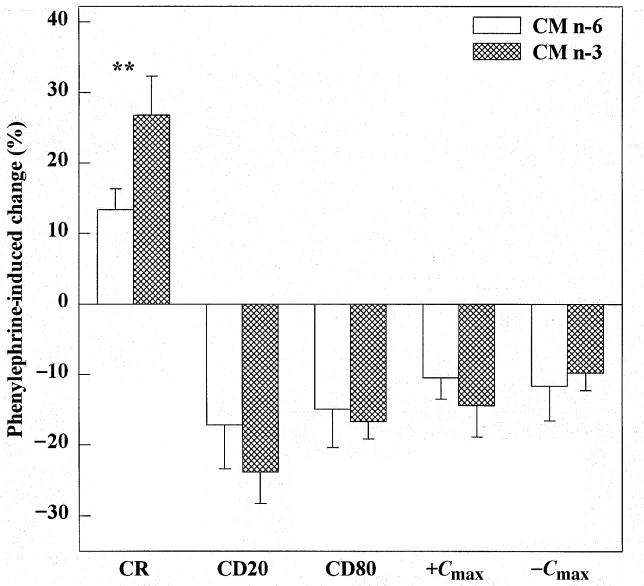

FIG. 5. Relation between PUFA composition and phenylephrine effects on the contractile parameters. Data are expressed as percentage of change induced by phenylephrine stimulation. Values are means ± SEM (n = 6). **P < 0.05. For abbreviations see Figures 1 and 3.

DISCUSSION

The present study was carried out to investigate the influence of long-chain PUFA on the contractility and adrenergic function of isolated rat CM in cell culture preparation. The membrane receptors, such as the enzymes and ionic channels, are presumably influenced by the composition of their lipid environment, which is linked to the fatty acid diet (10,11,27). To compare at a cellular level the consequences of two different fatty acid diets, two groups of CM, differing by the PUFA nature of their PL, were obtained by incubating the cultured cells in two different PUFA-enriched media. This protocol gave n-6 cells, which incorporated large amounts of AA, and n-3 cells that were characterized by the high amount of EPA, DPA, and DHA in their PL. The PL of these CM presented similar levels of SFA, MUFA, and PUFA but differed in their n-6/n-3 PUFA ratio. This ratio was close to 1 in n-3 cells, as in whole heart from rat after 8 wk of fish oil-enriched diet (8). In n-6 cells, the n-6/n-3 ratio reached 20.1 and was slightly higher than the value obtained from animals that were fed during 8 wk with a diet rich in sunflower seed oil.

The contractile parameters of cells maintained in standard medium were close to the values reported by Courtois *et al.* (17). In particular, the cultured ventricular muscle cells were not electrically driven, and in the present experiments, they exhibited spontaneous beating rates that were in the usual range as measured in this *in vitro* preparation (23). In addition, the rat ventricular myocytes in culture exhibited contraction velocities and contraction durations similar to those recorded in single isolated rat CM (26). Our observations showed that the modifications of the n-6/n-3 ratio of membrane PL do not affect spontaneous mechanical activity of CM in culture. This result was in agreement with data obtained from cultured myocardial cells that were only supplied with the C18 precursors of n-6 and n-3 fatty acids (17,18). These results favor the hypothesis that increasing the membrane level of long-chain PUFA does not modify the basal contractile function of CM. The inability of n-3 PUFA to influence the contractility of neonatal rat CM was already reported (28). Moreover, several studies in animals showed that modifications of the cardiac PUFA profile do not alter myocardial performances (9,29,30). These results from whole-heart preparation are in agreement with our data from individual CM, although no comparison between the n-6 and the n-3 series has been clearly explored *in vivo*.

β-Adrenoceptor function under physiological conditions was investigated by addition of isoproterenol, a β-adrenergic agonist. The results showed that modifications of the PL n-6/n-3 PUFA ratio had no direct effect on the inotropic response to isoproterenol stimulation. Indeed, the shortening and relaxation velocities ($+C_{max}$ and $-C_{max}$, respectively) were reduced after addition of isoproterenol, but the changes in these parameters did not differ significantly between the two cell groups. In opposition, this study provided additional data indicating that the rise in contraction rate after β-stimulation was influenced by the n-6/n-3 ratio because the positive chronotropic response was more pronounced in n-3 CM. This result extends the previous report of Courtois *et al.* (17)

and indicates that CM enriched with C18:n-3 PUFA exhibit enhanced positive chronotropy. Moreover, Fournier *et al.* (31) showed that, among the long-chain n-3 PUFA, DHA appeared to exert a greater efficiency because the isoproterenol-induced chronotropic response was improved in DHA-rich cells, compared to CM enriched with EPA + DPA. These overall data tend to demonstrate that the improved chronotropic response in n-3 cells, observed in our study, should be the direct consequence of great incorporation of DHA in the cell PL. To our knowledge, there are no comparable reports in the literature. It was previously suggested that fish oils or fish oil-derived PUFA do not modify the myocardial effects of adrenergic transmitters. In particular, Du *et al.* (32) reported that, in the perfused rat heart model, the functional response to β-agonist was not influenced by n-3 PUFA enrichment. Modifications of the n-6/n-3 PUFA ratio in the membrane PL could influence the β-adrenergic response by acting on the various steps of the β signaling pathway. Many studies have focused on the effects of PUFA on the binding characteristics of adrenoceptors, particularly in the heart, but these data are controversial. Grynberg *et al.* (33) showed that the β-receptor density of CM in culture was not influenced by the n-3 PL PUFA profile, but that a high content in DHA reduced the affinity for ligands. A similar observation was reported from rat heart after a fish oil-rich diet (11). Conversely, Wince *et al.* (34) showed that the β-adrenoceptor density and affinity were lowered in rat hearts that were submitted to a diet enriched in n-6 PUFA. The adenylate cyclase system in the cardiac membrane has been extensively studied and is considered sensitive to membrane lipid environment (35,36), although data were also discordant. Laustiola *et al.* (37) have observed that heart of rat fed a fish oil-rich diet displayed a low isoproterenol-induced AMPc accumulation. In opposition, increased adenylate cyclase activity was observed in rats and pigs subject to a similar diet (36).

In response to α-stimulation by PHE addition, an increased CR was observed in the two groups of cells. As for the β-stimulation, the positive chronotropic response obtained was greater in the n-3 cells. In opposition, the inotropic response to α-stimulation was not significantly influenced by the PUFA composition of PL because the shortening and relaxation times were reduced in a similar manner in the two groups of cells. Only a few studies have dealt with the influence of fatty acids on α-adrenergic function. Reibel *et al.* (16) showed that the cardiac inotropic response of isolated perfused rat heart to an α-agonist was reduced by a fish oil-rich diet, compared to a corn oil-rich diet. Our study demonstrated for the first time that the membrane long-chain PUFA composition can influence the α-adrenergic functional response. However, the ability of PUFA to alter the α response seems to depend on the extent of the change achieved in the long-chain PUFA. Grynberg *et al.* (33) did not show any difference in the positive chronotropic response between DHA-rich cells and (EPA + DPA)-rich cells. In this last study, however, the two groups of cells did not differ in their level of AA because they were enriched only in n-3 PUFA. In opposition, in our study, the proportion of AA was different in the n-3 and in the n-6 CM. Because AA is a major component of phosphatidylinositol (PI), which plays a key role in the α-adrenergic mechanism, the influence of PUFA on α-response, observed in the present study, could be linked to a change in the AA content of PI. Nevertheless, Meij *et al.* (38) observed that the total amount of inositol phosphates produced in rat heart cell culture was not affected by change in PL PUFA composition. In opposition, the treatment of cultured rat CM with linoleic acid (C18:2n-6) or EPA reduced the PHE-stimulated production of inositol phosphates (27). This reduction was suggested to be due to alterations of the activity of receptor-mediated phospholipase C (39).

These PUFA-induced modulations of α- and β-adrenergic responsiveness could be considered as functional correlates of the assumed dependence of the membrane functional proteins upon membrane dynamic properties (10). However, other mechanisms could be involved, such as alterations in intracellular steps of the transduction pathway, as suggested earlier (17,31). Moreover, the hypothesis of a specific influence of the eicosanoids cannot be excluded because Oudot *et al.* (40) observed that the production of prostacyclin by rat CM was different in EPA- and DHA-rich cells. Finally, the mechanisms by which dietary fish oils modulate the incidence of heart disease are still undefined. Dependency of the adrenergic receptor function upon the long-chain PUFA composition of the myocardial PL, here demonstrated under physiological conditions, may be a component of the cardioprotective effects of PUFA because these fatty acids may influence the susceptibility of the myocardium toward the catecholamines released during ischemia. Consequently, the influence of the fatty acid PL composition on the adrenergic function after hypoxia–reoxygenation will be explored in further studies.

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