Biochimica et Biophysica Acta 937 (1988) 347–358
Elsevier

BBA 73835

The influence of dietary lipid supplementation on cardiac β-adrenergic receptor adenylate cyclase activity in the marmoset monkey

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(Received 22 May 1987)

Key words: Dietary lipid; Membrane lipid; Adenylate cyclase; Lipid supplementation; Adrenergic receptor; Cardiac membrane; (Marmoset monkey)

Dietary lipid supplements high in either saturated fat derived from sheep kidney fat or unsaturated fat derived from sunflower seed oil, and a low mixed fat reference diet were fed to marmoset monkeys for 20 months and the effects on cardiac membrane lipid composition, and myocardial catecholamine-stimulated adenylate cyclase and β-adrenergic receptor binding activity were investigated. For cardiac membranes enriched for β-adrenergic binding activity, the dietary lipid treatment resulted in small changes in the proportion of saturated to unsaturated fatty acids and substantial changes in the $(n-6)$ to $(n-3)$ series of unsaturated fatty acids in the membrane phospholipids. The sheep kidney fat diet increased the cholesterol-to-phospholipid ratio in cardiac membranes in comparison to the other diets. This diet also significantly elevated basal and isoproterenol-, epinephrine- and norepinephrine-stimulated adenylate cyclase activity. The value of the dissociation constant (K_d) and the receptor number (B_{max}) for the binding of [^{125}I]ICYP to the β-adrenergic receptor was significantly reduced in marmosets fed the sheep kidney fat diet. These results suggest that dietary lipids can influence the activity of the β-adrenergic/adenylate cyclase system of the heart. Modulation of this transmembrane signalling system may be induced by changes in the properties of the associated membrane lipids, particularly by alteration in the membrane cholesterol-to-phospholipid ratio. This effect may be limited to those animal species in which the nature of the dietary fatty acid intake may be influencing cardiac membrane cholesterol homeostasis, which is in agreement with previous results in rats following dietary cholesterol supplementation (McMurchie et al. (1987) Biochim. Biophys. Acta 898, 137–153). ICYP, (−)-iodocyanopindolol.

Introduction

Many of the processes controlling the contractile cycle of the mammalian heart are performed by enzymes, receptors and ion channels which are associated with various cardiac membranes, particularly the sarcolemmal membrane. Included in this category is the β-adrenergic/adenylate cyclase

Abbreviations: EGTA, ethyleneglycol bis(β-amino-ethyl ether)-*N*,*N*′-tetraacetic acid; ICYP, (−)-iodocyanopindolol; BHT, butylated hydroxytoluene. Diets: REF, reference, standard laboratory diet; SKF, sheep kidney fat-supplemented diet; SSO, sunflower seed oil-supplemented diet.

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system which when activated by catecholamines, increases the intracellular level of cyclic AMP (cAMP) [1,2]. By means of a cAMP-dependent protein kinase, cAMP is believed to lead to the phosphorylation of the slow calcium channel and subsequent enhancement of the number of calcium channels available for Ca^{2+} entry during membrane depolarization [3,4]. Resulting increases in the level of intracellular calcium ions contribute to the enhanced inotropic and chronotropic response of the heart in response to increased sympathetic drive.

The β-adrenergic/adenylate cyclase system consists of a receptor unit capable of binding adrenergic ligands with high affinity, a stimulatory guanine nucleotide regulatory protein (G_s), and the catalytic unit which converts ATP to cAMP [5,6]. The function of this enzyme-receptor complex, and indeed hormone-sensitive adenylate cyclases in general, has now been shown to depend on the maintenance of a suitable membrane environment [7]. Changes in those lipid components of the membrane with which this system is associated, can dramatically influence many properties of this signalling mechanism [8,9]. This latter property has prompted many investigations into the effects of dietary lipids on hormone-activated adenylate cyclases [10–13]. Relatively few studies have focussed on the possible effects of dietary lipids on the catecholamine-stimulated adenylate cyclase system of the heart, and we are not aware of reports on the effects of dietary lipids on this system using non-human primates as experimental animal models.

We have recently demonstrated that dietary cholesterol supplementation influences the activity of the β-adrenergic/adenylate cyclase system of the rat heart, mediated by changes in the membrane cholesterol-to-phospholipid ratio [14]. Dietary fatty acid supplementation has been reported both to influence [15] and have no effect [14] on this hormone-sensitive system in rat heart. In addition to these findings, we have documented the changes that occur in cardiac membrane lipid composition of rats and marmoset monkeys when these animals are fed diets rich in saturated fats derived from a sheep kidney fat supplement, or in polyunsaturated fatty acids provided by supplementation with sunflower seed oil [16–24]. We have shown that such lipid-supplemented diets influence cardiac contractility and arrhythmogenesis [25–27], cardiac mitochondrial oxidation [21] and membrane-associated ATPase activity [16,28].

Of particular relevance is our finding that ventricular tachyarrhythmias in rats [25,27] and marmoset monkeys [26] can be substantially attenuated by a sunflower seed oil dietary supplement. By contrast, the saturated fatty acid-enriched diet heightened vulnerability to arrhythmogenesis. These observations are pertinent to the recent demonstration of an inverse correlation between the prevalence of coronary heart disease mortality and the polyunsaturated to saturated fatty acid ratio of adipose fat [29]. In this study we report the effects of dietary fatty acids on the cardiac β-adrenergic/adenylate cyclase system of the marmoset monkey. The experimental findings suggest that this system can be modulated by dietary lipids and this may in part be responsible for dietary lipid effects on the vulnerability of the heart to arrhythmogenesis.

Materials and Methods

Marmosets. Adolescent male common cotton-eared marmosets (*Callithrix jacchus jacchus*) approx. 12 to 14 months of age at the start of the experiment were paired for optimal growth and social behaviour and kept in aluminium alloy marmoset cages in a room with fluorescent light and 30 min of ultraviolet irradiation daily. The temperature was maintained at 26 °C and the humidity at approx. 50%. Marmosets were maintained on the various dietary lipid regimes described below for a period of 20 months. Animals were killed under anaesthesia (Ketamine, 100 mg/kg i.m. (Ketalar, Parke Davis, Australia) and anaesthetic diethyl ether). The heart was then removed for immediate preparation of membrane fractions. Marmosets weighed an average of 318 g at the start of the experiment and 342 g at the finish of the experiment, and there was no significant difference in the weight gain between dietary groups.

Marmoset diets. One group of marmosets was fed a standard commercial diet consisting of a 1 : 1 mixture of Arnott Harper's (Adelaide, Australia) greyhound chow and Milling Industries (Adelaide,

Australia) primate meal. The overall composition of this diet has previously been described [30], and on analysis contained 4% (w/w) total fat. The fatty acid composition of this diet which was designated the reference (REF) diet, is shown in Table I. A second group of marmosets was fed the above diet supplemented at the time of repelleting with sunflower seed oil (Nuttelex, Melbourne, Australia) to give a total of 19% (w/w) fat (designated the SSO diet). A third group of marmosets was fed the reference diet supplemented with sheep kidney (perirenal) fat, a natural source of both saturated and monounsaturated fat, to give a total of 19% (w/w) fat (designated the SKF diet). The various fat supplements were added to the crushed REF diet and thoroughly mixed before the respective diets were repelleted. The amount of cholesterol present in these three diets was less than 0.04% (w/w) [21]. The fatty acid composition of the sunflower seed oil and sheep kidney fat used in the diet preparations has been reported previously [18,20,21,23] and the fatty acid composition of the final fabricated marmoset diets is shown in Table I. These diets were supplied ad libitum.

TABLE I

FATTY ACID COMPOSITION OF FABRICATED MARMOSET DIETS

Fatty acids are designated by the number of carbon atoms followed by the number of double bonds. The particular unsaturated fatty acid series for each unsaturated fatty acid is shown as $(n-x)$ where (x) refers to the position of the first double bond counting from the terminal methyl group of the fatty acid. REF, reference, standard laboratory diet; SKF, sheep kidney fat-supplemented diet; SSO, sunflower seed oil-supplemented diet. Trace amounts (tr., less than 0.2%) were also detected for the fatty acids 20:0, 22:0 and 22:6$(n-3)$.

Fatty acid	REF	SKF	SSO
14:0	2.2	2.5	0.5
16:0	25.0	24.1	12.0
16:1$(n-7)$	3.7	1.8	tr.
18:0	14.4	29.6	7.9
18:1$(n-9)$	33.9	32.6	26.3
18:2$(n-6)$	19.2	8.6	52.5
18:3$(n-3)$	1.5	0.9	0.7
Unsatd.	58.3	43.9	79.5
Satd.	41.6	56.2	20.4
Polyunsatd./Satd.	0.50	0.17	2.61
$(n-6)/(n-3)$	12.8	9.5	75.0

Preparation of cardiac membrane fractions. Ventricular tissue from each marmoset heart was chopped and rinsed in ice-cold isolating medium comprising 250 mM sucrose/20 mM Tris/1 mM EDTA/1 mM $MgCl_2$ (pH 7.4) and then homogenized in 40 ml of the above medium using a Polytron tissue homogenizer (Kinematica, Switzerland) at setting 4 for three bursts each of 30 s. A P0–500g, low-speed membrane pellet and a P6000g–46 000g post-mitochondrial membrane fraction (high-speed pellet), were isolated by differential centrifugation as previously described [14].

Adenylate cyclase assay. Adenylate cyclase (ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1) activity was measured as previously described [14]. Assays performed in triplicate, were initiated by the addition of 40 μg of membrane protein from the low-speed membrane pellet (P0–500g).

β-Adrenergic receptor binding assay. Cardiac β-adrenergic receptor binding activity was determined using the P6000g–46 000g membrane fraction and the β-adrenergic radioligand, ICYP, with specific binding being determined in the presence of 10^{-5} M propranolol as previously described [14]. Assays, done in triplicate, were initiated by the addition of ICYP and binding was performed using 20 μg membrane protein per assay.

Protein determination. Values for the membrane protein content of cardiac membrane preparations were determined by the method of Lowry et al. [31], after solubilization of the membranes in 0.1 M NaOH and 1% (w/v) SDS, and using bovine serum albumin as standard.

Cardiac membrane fatty acid analysis. Fatty acid analysis was performed on the phospholipids isolated from the total lipids extracted from the P6000g–46 000g (high-speed membrane fraction) as previously described [14,23]. The fatty acids present in the various dietary lipid supplements were analysed similarly.

Cardiac membrane phospholipid and cholesterol determination. The cholesterol-to-phospholipid (mol/mol) ratio was determined on total lipid extracts of the P6000g–46 000g cardiac membrane fraction. Membrane total lipids were extracted as described above for fatty acid analysis. Membrane phospholipid content was determined using the

method of Bowyer and King [32], with egg-yolk phosphatidylethanolamine as standard. For membrane cholesterol determination, 0.1 ml of 10M KOH was added to 60 μg of the membrane total lipid extract in 1.5 ml of 95% (v/v) ethanol and the mixture was heated at 60 °C for 25 min for hydrolysis of cholesterol esters. Campesterol was added before hydrolysis as a recovery marker. These and samples for plasma cholesterol determination were analysed using a Hewlett Packard 5710 gas chromatograph fitted with a glass column packed with 1% OV-101 on Gas Chrom. Q silica gel (Alltech). Cholestane was used as the internal standard.

Chemicals. 1-Isoproterenol; (±)-propranolol; (−)-epinephrine; (−)-arterenol; ATP disodium; 3-isobutyl-1-methylxanthine; creatine phosphate, disodium; cholesterol; cholestane; campesterol; creatine phosphokinase, rabbit muscle; and bovine serum albumin, fraction V were supplied by Sigma. Cyclic AMP, free acid; dithiothreitol; GTP, dilithium, were supplied by Boehringer-Mannheim. Forskolin was from Calbiochem. (−)-[^{125}I]-Iodocyanopindolol, 2200 Ci/mmol, over 99% pure; [α-^{32}P]ATP tetra(triethylammonium)salt, 3000 Ci/mmol, over 99% pure; [2,8-^{3}H]cAMP ammonium salt, 31.1 Ci/mmol, over 99.5% pure, were supplied by New England Nuclear. Solvents were of highest analytical grade and were redistilled and gassed with N_2 before use. All other chemicals were of the highest reagent grade available.

Results

The fatty acid compositions of the fabricated marmoset diets are shown in Table I. Addition of sheep kidney fat to the marmoset reference diet decreased the ratio of polyunsaturated to saturated fatty acids mainly by increasing the proportion of 18:0 and decreasing the proportion of 18:2 ($n-6$). Addition of the sunflower seed oil to the reference diet markedly increased the polyunsaturated to saturated fatty acid ratio and the $(n-6)/(n-3)$ polyunsaturated fatty acid ratio.

Membrane lipid analysis

Membrane lipid analysis was performed on the high-speed cardiac membrane preparation (i.e., P6000g–46 000g) obtained by centrifuging the post-mitochondrial supernatant at 46 000g. Previous studies [33] have shown that this particular membrane fraction exhibited the greatest number of β-adrenergic receptors (identified by [^{125}I]iodocyanopindolol binding). The effect of the various lipid supplemented diets on the fatty acid composition of marmoset heart membrane phospholipids is shown in Table II. Feeding the sheep kidney fat-supplemented diet did not significantly alter the proportion of saturated, unsaturated or polyunsaturated fatty acids in the membrane phospholipids in comparison to feeding the reference diet. However, changes in the proportion of some of the individual polyunsaturated fatty acids were evident. There was a decrease in both 18:2($n-6$) and 20:4($n-6$), and an increase in 22:5($n-3$) which led to a decrease in the $(n-6)/(n-3)$ ratio. Feeding the sunflower seed oil-supplemented diet decreased the proportion of saturated fatty acids and increased the proportion of unsaturated and polyunsaturated fatty acids in comparison to the other two dietary treatments, resulting in an elevation in the ratio of polyunsaturated to saturated fatty acids. Furthermore, the sunflower seed oil-supplemented diet increased the proportion of the ($n-6$) series of polyunsaturated fatty acids and decreased those of the ($n-3$) series, resulting in a considerable increase in the $(n-6)/(n-3)$ ratio in comparison to that obtained when feeding the other two diets. For the ($n-6$) series of polyunsaturated fatty acids, the sunflower seed oil-supplemented diet resulted in an increase in the proportion of 18:2($n-6$) and a decrease in 20:4($n-6$). This latter effect has previously been observed in marmoset heart mitochondrial membranes following similar dietary lipid treatment [24].

The cholesterol-to-phospholipid ratio of cardiac membranes was also determined using lipids extracted from the high-speed (P6000g–46 000g) membrane pellet. The effect of the various dietary lipid supplements on both the cholesterol-to-phospholipid ratio and the level of plasma cholesterol, are shown in Table III. The value for the cholesterol-to-phospholipid ratio in hearts from marmosets fed the sheep kidney fat supplemented diet was significantly higher than the value obtained for the sunflower seed oil-supplemented

TABLE II

FATTY ACID COMPOSITION OF MARMOSET HEART MEMBRANE PHOSPHOLIPIDS AFTER 20 MONTHS DIETARY LIPID SUPPLEMENTATION

Fatty acids are the mean relative percentage ± S.E. for single heart preparations from $n = 6$ REF, $n = 6$ SKF and $n = 5$ SSO dietary animals. Fatty acid analysis was performed on the total phospholipids of the high-speed P6000g–46000g cardiac membrane fraction. The particular unsaturated fatty acid series for the majority of the unsaturated fatty acids is also shown. The unsaturation index (U.I.) is Σ_{a-1}^{k} (number of double bonds in a)×(% occurrence of a) for each fatty acid of k fatty acids. tr., (trace) present at less than 0.2%. DMA, dimethyl acetal derivative. The significance of differences between the major fatty acids and the computational parameters for each dietary group were determined by Student's t-test with (–), not determined, and n.s., not significant.

Fatty acid		Diet			Significance		
		REF	SKF	SSO	REF vs. SKF	REF vs. SSO	SKF vs. SSO
	14:0	0.5±0.1	0.5±0.1	0.3±0.1	–	–	–
	15:0	tr.	tr.	tr.	–	–	–
DMA	16:0	1.0±0.2	0.7±0.2	0.5±0.1	–	–	–
	16:0	12.9±0.9	10.8±0.6	9.0±0.5	n.s.	$P < 0.02$	$P < 0.05$
	16:1($n-7$)	1.4±0.1	2.0±0.5	1.2±0.1	–	–	–
	17:0	0.4±0.1	0.6±0.1	0.3±0.1	–	–	–
DMA	18:0	1.8±0.3	2.1±0.4	0.8±0.3	–	–	–
	18:0	16.1±0.6	17.3±0.5	18.3±0.8	n.s.	n.s.	n.s.
	18:1($n-9$)	15.8±1.2	17.6±1.0	11.8±0.8	n.s.	$P < 0.05$	$P < 0.005$
	18:2($n-6$)	23.7±0.5	19.2±0.4	40.0±2.2	$P < 0.001$	$P < 0.001$	$P < 0.001$
	18:3($n-6$)	0.3±0.1	0.8±0.2	1.0±0.6	–	–	–
	18:3($n-3$)	0.4±0.1	0.7±0.1	0.4±0.1	–	–	–
	20:0	tr.	0.2±0.1	0.3±0.1	–	–	–
	20:1	0.5±0.1	0.9±0.2	0.3±0.1	–	–	–
	20:2($n-6$)	0.7±0.1	1.2±0.3	1.3±0.4	–	–	–
	20:3($n-6$)	0.8±0.1	1.3±0.3	0.9±0.4	–	–	–
	20:4($n-6$)	15.2±0.8	12.9±0.5	7.8±0.4	$P < 0.05$	$P < 0.001$	$P < 0.001$
	22:1/20:5($n-3$)	0.8±0.1	1.8±0.6	0.3±0.1	–	–	–
	22:4($n-6$)	0.5±0.1	0.7±0.1	1.1±0.3	–	–	–
	24:0	1.0±0.1	0.8±0.1	1.3±0.3	–	–	–
	22:5($n-3$)	2.4±0.2	3.1±0.1	1.8±0.3	$P < 0.02$	n.s.	$P < 0.005$
	22:6($n-3$)	3.5±0.5	4.5±0.3	2.2±0.5	n.s.	n.s.	$P < 0.005$
Satd. (S)		33.9±0.3	33.2±0.7	30.8±0.5	n.s.	$P < 0.001$	$P < 0.05$
Unsatd.		66.1±0.3	66.8±0.7	69.2±0.5	n.s.	$P < 0.001$	$P < 0.05$
Polyunsatd. (P)		48.3±1.3	45.4±0.3	54.8±0.9	n.s.	$P < 0.005$	$P < 0.001$
($n-6$)		41.2±1.0	36.2±0.5	51.0±0.9	$P < 0.005$	$P < 0.001$	$P < 0.001$
($n-3$)		7.1±0.6	10.1±0.9	4.8±0.8	$P < 0.02$	$P < 0.05$	$P < 0.005$
DMA		2.7	2.8	1.2	–	–	–
P/S		1.42	1.37	1.78	–	–	–
($n-6$)/($n-3$)		5.80	3.56	10.64	–	–	–
U.I.		168	169	159	–	–	–

group and also higher than that of the reference group. Plasma cholesterol levels were also elevated in marmosets fed the sheep kidney fat-supplemented diet and decreased in the sunflower seed oil group relative to the reference group.

Adenylate cyclase studies

The effect of dietary lipid supplementation on adenylate cyclase activity and its stimulation by catecholamines and other activators was studied using the low-speed (P0–500g) cardiac membrane fraction. From other studies on rat and marmoset heart adenylate cyclase [33], this particular cardiac membrane fraction was found to exhibit the greatest stimulation by the β-adrenergic agonist, isoproterenol, and to also exhibit the highest level of

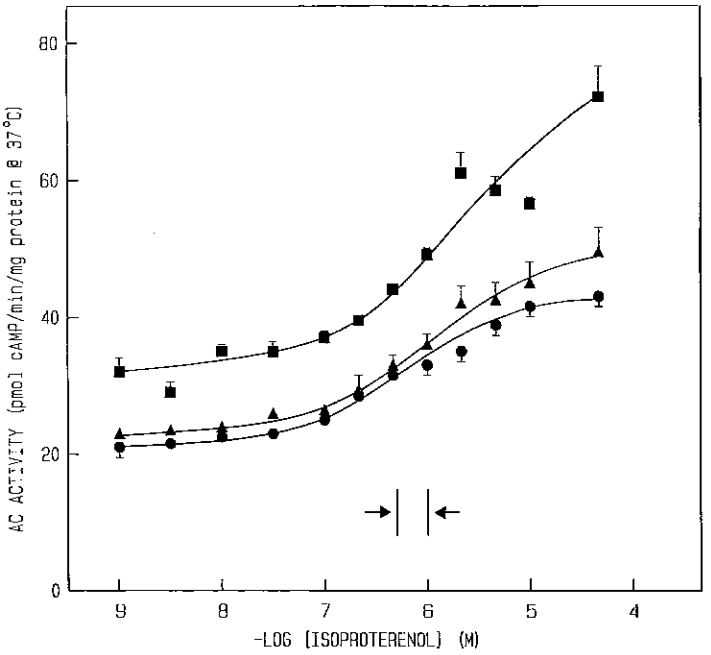

Fig. 1. Isoproterenol dose-response curves for marmoset heart membrane adenylate cyclase (AC) activity after 20 months dietary lipid supplementation. Data are shown as the mean ± S.E. for the dietary groups; ●, REF; ■, SKF; ▲, SSO. The ED_{50} values were determined from normalized dose-response curves and were $6.57 \cdot 10^{-7}$ M (REF), $9.62 \cdot 10^{-7}$ M (SKF) and $7.64 \cdot 10^{-7}$ M (SSO) with the range for these values indicated on the figure. Significant differences between the REF and SKF dietary groups were apparent at isoproterenol concentrations of $3.16 \cdot 10^{-8}$ M, $2.15 \cdot 10^{-6}$ M and $5 \cdot 10^{-5}$ M ($P < 0.05$); $4.65 \cdot 10^{-6}$ M ($P < 0.02$); 10^{-8} M, 10^{-7} M and 10^{-5} M ($P < 0.01$), and at $2.15 \cdot 10^{-7}$ M, $4.65 \cdot 10^{-7}$ M and 10^{-6} M ($P < 0.005$). Significant differences between the SKF and SSO groups were apparent at 10^{-8} M and 10^{-7} M ($P < 0.02$). No significant difference was apparent between the REF and SSO dietary groups at any of the isoproterenol concentrations tested.

forskolin-stimulated adenylate cyclase activity.

Dose response curves (plotted as specific activity) for isoproterenol stimulation of adenylate cyclase activity in heart preparations from marmoset monkeys fed the various lipid-supplemented diets are shown in Fig. 1. It is evident that adenylate cyclase activity from the hearts of marmosets maintained on the sheep kidney fat diet exhibited a higher basal and isoproterenol-stimulated activity in comparison to the other two dietary groups. These differences were statistically significant for a number of isoproterenol con-

TABLE III

CARDIAC MEMBRANE CHOLESTEROL-TO-PHOSPHOLIPID RATIO AND PLASMA CHOLESTEROL LEVELS IN MARMOSET MONKEYS AFTER 20 MONTHS DIETARY LIPID SUPPLEMENTATION

Data are presented as the mean ± S.E. for $n = 5$ REF, $n = 6$ SKF and $n = 5$ SSO dietary supplemented animals. Cholesterol-to-phospholipid ratios were determined from the total lipids extracted from the P6000g–46 000g membrane fraction as described in Materials and Methods. Differences between means were significant at $P < 0.05$ for SKF vs. SSO by Student's t-test for both the membrane cholesterol-to-phospholipid ratio and plasma cholesterol levels.

Parameter	Diet		
	REF	SKF	SSO
Cardiac membrane cholesterol to phospholipid (mol/mol)	0.238 ± 0.017	0.278 ± 0.020	0.221 ± 0.010
Plasma cholesterol (mg/100 ml)	137 ± 8	145 ± 6	123 ± 7

TABLE IV

ADENYLATE CYCLASE ACTIVITY OF MARMOSET HEART MEMBRANES AFTER 20 MONTHS DIETARY LIPID SUPPLEMENTATION

Data are shown as pmol cAMP/min per mg protein at 37 °C and are expressed as the mean ± S.E. for $n = 5$ animals in the REF group, $n = 4$ animals in the SSO diet group and $n = 5$ animals in the SKF diet group. ΔcAMP is the activity in the presence of $5 \cdot 10^{-5}$ M isoproterenol minus the basal activity. The significance of differences in adenylate cyclase activity between the dietary groups for the additions shown was determined by Student's t-test, with n.s., not significant.

Addition	Diet			Significance		
	REF	SKF	SSO	REF vs. SKF	REF vs. SSO	SKF vs. SSO
Basal	34.0 ± 1.1	43.2 ± 1.8	36.2 ± 1.9	$P < 0.005$	n.s.	$P < 0.05$
Propranolol (10^{-4} M)	43.6 ± 3.1	55.6 ± 2.4	50.0 ± 1.5	$P < 0.02$	n.s.	n.s.
Isoproterenol (10^{-8} M)	35.5 ± 1.3	42.2 ± 2.4	34.5 ± 1.7	$P < 0.05$	n.s.	$P < 0.05$
Isoproterenol ($5 \cdot 10^{-5}$ M)	54.8 ± 2.6	72.2 ± 2.7	60.9 ± 3.6	$P < 0.005$	n.s.	$P < 0.05$
ΔcAMP	21.1 ± 2.3	31.9 ± 2.5	26.6 ± 2.5	$P < 0.005$	n.s.	n.s.
Isoproterenol ($5 \cdot 10^{-5}$ M) + propranolol (10^{-4} M)	45.6 ± 3.4	58.6 ± 3.0	47.7 ± 3.1	$P < 0.02$	n.s.	$P < 0.05$
Epinephrine ($5 \cdot 10^{-5}$ M)	54.1 ± 2.9	63.5 ± 2.6	54.6 ± 2.4	$P < 0.05$	n.s.	$P < 0.05$
Norepinephrine ($5 \cdot 10^{-5}$ M)	49.9 ± 3.6	63.5 ± 2.6	46.8 ± 2.0	$P < 0.02$	n.s.	$P < 0.005$
NaF (10 mM)	116 ± 5	134 ± 6	124 ± 4	$P < 0.05$	n.s.	n.s.
Forskolin (100 μM)	400 ± 11	501 ± 23	451 ± 35	$P < 0.005$	n.s.	n.s.

centrations, as indicated in the figure legend. The ED_{50} values for isoproterenol stimulation of adenylate cyclase were between $6.57 \cdot 10^{-7}$ M and $9.62 \cdot 10^{-7}$ M and were not influenced by the dietary lipid treatments. The effect of the various dietary lipid treatments on other conditions associated with the activation of marmoset heart adenylate cyclase, as well as some results derived from the isoproterenol data of Fig. 1, are shown in Table IV. For all the conditions shown, the activity of adenylate cyclase in the reference group was not statistically different from that in the sunflower seed oil group. However the activity in the sheep kidney fat-supplemented group was significantly higher in comparison to the reference group. Although the sheep kidney fat dietary group exhibited higher adenylate cyclase activity than the sunflower seed oil group for all the conditions shown in Table IV, a statistically significant difference was not apparent for all conditions. When a comparison between the sheep kidney fat and reference groups was made, the greatest increase was 51% for the value of the ΔcAMP activity. Addition of $1 \cdot 10^{-4}$ M propranolol alone resulted in an increase (28% to 38%) in adenylate cyclase activity in marmoset heart; a result previously observed [33]. The activity in the presence of propranolol (and with isoproterenol in the presence of excess propranolol), was increased in the sheep kidney fat group relative to the other two dietary groups. Increased adenylate cyclase activity was also evident when using either epinephrine or norepinephrine at concentrations of $5 \cdot 10^{-5}$ M. The increase in NaF- and forskolin-stimulated adenylate cyclase activity in the sheep kidney fat-dietary group was only statistically significant in comparison to the reference group and resulted in an increase of 15% and 25%, respectively.

β-Adrenergic receptor binding studies

Representative Scatchard plots for the binding of ICYP to marmoset heart membrane preparations are shown in Fig. 2. Binding assays were performed using the high-speed pellet (P6000g–46000g) from the marmoset heart, as this particular membrane fraction has previously been shown to exhibit the greatest number of β-adrenergic binding sites in comparison to other cardiac membrane fractions prepared by differential centrifugation [33]. A summary of the parameters obtained from the binding experiments is shown in Table V. The K_d value for ICYP binding in the marmoset was lower for the sheep kidney fat dietary group relative to the other two

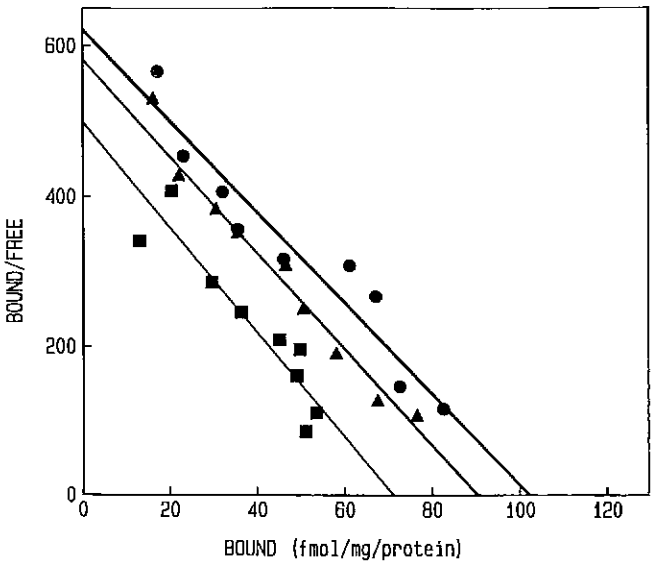

Fig. 2. Binding of the β-adrenergic ligand [^{125}I]iodocyanopindolol to marmoset heart membranes after 20 months dietary lipid supplementation. Scatchard plots were generated by pooling data from $n = 5$ REF (●), SKF (■) and SSO (▲) P6000g–46000 ig cardiac membrane preparations with one heart per preparation. The regression coefficient for each line was more than −0.95. From the pooled data the K_d (nM) and B_{max} (fmol/mg protein) values were 0.205, 102 (REF); 0.130, 71 (SKF); 0.180, 93 (SSO).

dietary groups, although statistical significance was only achieved in comparison to the sunflower-seed oil group. The receptor number (B_{max}) was decreased in the sheep kidney fat group in comparison to the other two dietary groups, as can also be seen in the Scatchard plot for ICYP binding to marmoset heart membranes (Fig. 2).

Discussion

Although both sheep kidney fat and sunflower-seed oil treatments resulted in changes in the fatty acid profile of marmoset cardiac membrane phospholipids, the activity of the catecholamine-stimulated adenylate cyclase system was only increased as a result of the dietary sheep kidney fat treat-

TABLE V

BINDING OF [^{125}I]IODOCYANOPINDOLOL TO THE β-ADRENERGIC RECEPTOR OF MARMOSET HEART MEMBRANES AFTER 20 MONTHS DIETARY LIPID SUPPLEMENTATION

The dissociation constant (K_d) and the receptor number (B_{max}) were calculated from individual Scatchard plots as described in Materials and Methods with regression coefficients greater than −0.95 and specific binding of more than 85% (at K_d) as determined by competition against 10^{-5} M propranolol. Data are shown as the mean ± S.E. for $n = 5$ animals in each dietary group. Binding experiments were performed on P6000g–46000g membrane preparations. The significance of differences between means was determined by Student's t-test. *, $P < 0.05$, SKF vs. SSO; **, $P < 0.02$, SKF vs. REF.

Binding parameter	Diet		
	REF	SKF	SSO
K_d (nM)	0.205 ± 0.042	0.130 ± 0.014 *	0.180 ± 0.016
B_{max} (fmol/mg)	105 ± 11	68 ± 7 **	96 ± 10

ment. This increase in adenylate cyclase activity may be related to the cholesterol-to-phospholipid ratio, which was elevated in cardiac membranes of marmosets fed the high saturated fatty acid, sheep kidney fat-supplemented diet relative to animals fed the other diets. In this regard, the results differ markedly from those observed for rat heart, in which dietary sheep kidney fat or sunflower seed oil supplementation did not significantly influence catecholamine-stimulated (β-adrenergic) adenylate cyclase activity [14]. However, dietary cholesterol supplementation in the rat has been shown to influence dramatically the cardiac β-adrenergic/adenylate cyclase system, possibly by changing the membrane cholesterol-to-phospholipid ratio [14]. Thus, the different response of the cardiac β-adrenergic/adenylate cyclase system of the rat and the marmoset monkey to dietary fatty acid supplementation may reside in the ability of such diets to perturb membrane physical properties via changes in the membrane cholesterol-to-phospholipid ratio. This biophysical effect may in part be due to the different effect of these fatty acid supplements on plasma cholesterol levels in the rat and the marmoset monkey [14].

The results of this study and those previously performed in the rat [14] suggest that changes in the total phospholipid fatty acid composition of cardiac membranes per se were not sufficient to influence membrane-associated catecholamine-stimulated adenylate cyclase activity. This is supported by the fact that the feeding of the sheep kidney fat diet did not substantially alter the proportions of saturated or unsaturated fatty acids in the cardiac membrane phospholipids in either the marmoset or the rat [14]. Furthermore, in both species, the dietary treatments had similar effects on the ratio of the membrane $(n-6)$ to $(n-3)$ series of polyunsaturated fatty acids, the value of which was elevated with the sunflower seed oil diet and reduced with the sheep kidney fat diet relative to the low mixed-fat reference diet. These dietary treatments have also been shown not to alter significantly cardiac membrane phospholipid headgroup composition in the rat [19,23] or the marmoset [22]. Therefore, the failure of the saturated fat diet to alter membrane lipid saturation/unsaturation or phospholipid headgroup composition substantially, together with the similarity of changes in the $(n-6)/(n-3)$ ratio of polyunsaturated fatty acids in the cardiac membranes of both animals, would suggest that the major membrane lipid modification which was unique to the marmoset during this diet was the altered cholesterol-to-phospholipid ratio. This does not exclude the possibility that the sheep kidney fat diet stimulated the adenylate cyclase system through an effect not specifically measured in this study. Included in this category may be some particular change or combination of changes in the acyl fatty acid composition of one or more of the individual membrane phospholipid species. Although these compositional measurements have been made in both rats and marmosets following dietary lipid supplementation of the type described in this study [22,23], the changes are complex and are not readily reconcilable with the different responses observed in cardiac adenylate cyclase activity between these animal species following dietary lipid supplementation.

The possibility that the lateral mobility of some or all of the components of hormone-sensitive adenylate cyclases is involved in some manner with the process of transmembrane signalling [34,35], has led to the suggestion, which has now been supported by much experimental evidence, that hormone-sensitive adenylate cyclases are sensitive to certain compositional characteristics of their host membrane lipids. For hormone-sensitive adenylate cyclases this has been confirmed by a number of techniques such as in vivo modification of membrane lipid composition by dietary lipid supplementation [10–15], modification of the membrane lipid composition of cultured cell lines [36,37], in vitro lipid modification of isolated cell membrane preparations containing hormone-receptor adenylate cyclase components [38,39], reconstitution experiments [40], and by introducing lipid-perturbating agents into membranes [41,42]. Furthermore, hormone-sensitive adenylate cyclases have been shown to be particularly sensitive to changes in membrane cholesterol, especially alterations in the value of the cholesterol-to-phospholipid ratio [12,14,38,43,44]. This may indicate that the effect is mediated by altered physical properties of the membrane, although the possibility of a direct effect of cholesterol on membrane proteins cannot be ruled out [45].

The possibility that increased acyl fatty acid chain order may have been responsible for the increased adenylate cyclase activity would require direct biophysical measurements using for example, fluorescent probes. A thermodynamic interpretation based on increased enthalpy and entropy of enzyme activation accompanying increased membrane lipid order with subsequent stabilization of the enzyme in a more reactive conformation has been proposed [43]. However, the actual mechanism whereby an elevated (cardiac) membrane cholesterol-to-phospholipid ratio, and presumably increased membrane lipid order might lead to an increased catecholamine-stimulated adenylate cyclase activity is clearly not known.

In our study, the increased catecholamine-stimulated adenylate cyclase activity was accompanied by a significant increase in β-adrenergic receptor affinity and a decrease in receptor number. The decrease in receptor number may be a reflection of β-adrenergic receptor down-regulation [1] in response to the increased adenylate cyclase activity in marmosets fed the high saturated fatty acid, sheep kidney fat-supplemented diet. Indeed, for rats fed cholesterol supplemented diets, which significantly increase catecholamine-stimulated adenylate cyclase activity, there is an equally dramatic decrease in β-adrenergic receptor number with no accompanying change in receptor affinity [14]. The significance of the change in β-adrenergic receptor affinity in this present study in regard to altered adenylate cyclase activity is not known. However in the study by Scarpace et al. [39], in vitro incorporation of cholesteryl hemisuccinate did not alter β-adrenergic receptor affinity. In addition, β-adrenergic receptor affinity was not altered in quail erythrocyte membranes following dietary cholesterol supplementation, although isoproterenol-stimulated cAMP accumulation in erythrocytes from cholesterol-fed quails was significantly elevated [13]. Loh and Law [8] conclude that the role of lipids in modulating β-adrenergic receptor binding is not at all conclusive, whereas the involvement of membrane lipids and their modulating effects on the coupling of the β-adrenergic receptor complex to the effector, is more clear-cut. Thus it is likely that the effects observed in the present study were mediated by receptor-transducer alterations and that the reduction in β-adrenergic receptor number did not 'protect' against enhanced catecholamine-stimulated adenylate cyclase activity.

As has already been reported using rats [20,46] and marmosets [24], considerable homeostasis is observed in the membrane lipid composition, despite considerable differences in the nature of the dietary lipid intake. This is particularly evident in the maintenance of a relatively constant proportion of membrane lipid saturation and unsaturation. We have not previously reported the effects of dietary lipid supplementation on this particular cardiac membrane preparation. As described earlier, this membrane preparation was chosen on the basis of a compromise between the limited amount of cardiac tissue available from the marmoset monkey, and the need to have a membrane preparation containing a sufficient number of β-adrenergic receptors to undertake meaningful binding experiments [33]. However, the nature of the changes in the cardiac membrane phospholipid fatty acids when feeding identical fatty acid-supplemented diets is qualitatively similar to that described previously for heart mitochondrial membranes from the marmoset [21,24] and, indeed, the rat [16,17,20]. The change in the value of the $(n-6)$ to $(n-3)$ polyunsaturated fatty acid ratio is in accord with changes in the flux of the various products of these non-interconvertable fatty acid pathways for chain elongation and desaturation in response to variable levels of precursor fatty acids in the particular dietary lipid supplement. As mentioned previously, the proportion of cardiac phospholipids is not significantly altered by these dietary lipid treatments [22,23].

The results presented in this study clearly show that dietary fatty acid supplementation can significantly alter cardiac catecholamine-stimulated adenylate cyclase activity in the marmoset monkey. The relevance of these findings in the marmoset to cardiac tachyarrhythmias and sudden cardiac death in humans is intriguing. We have already reported the much higher incidence of catecholamine-induced ventricular arrhythmia in marmosets fed sheep kidney fat than in those fed sunflower-seed oil [26]. There is compelling evidence in human populations that diets richer in plant than in animal derived foods are associated with

lower cardiac associated mortality. In both the Western Electric (Chicago) Heart Study [47] and the Ireland-Boston Diet Study [48], a higher intake of polyunsaturated fatty acids and a lower intake of saturated fatty acids were significantly correlated with fewer cardiac deaths. Although no single dietary intervention trial has been conclusive, taken together, there is a strong indication that recurrence of cardiac events is lessened by reducing the saturated fatty acid intake [49]. At the biochemical level it is therefore tempting to speculate that dietary-induced alterations in certain transmembrane signalling mechanisms critical to the control of cardiac rhythmicity and contractility may in part be involved in the genesis of cardiac dysfunction.

Acknowledgements

We thank Dr. G. McIntosh, Mr. J. Greenfield and Mr. J. Cooper for care of the experimental animals and Ms. S. Crouch for technical assistance. We thank Dr. R. Gibson for assistance with the fatty acid analysis. This work was funded in part from a grant-in-aid from the National Heart Foundation of Australia (to E.J.M.).

References

1 Lefkowitz, R.J., Stadel, J.M. and Caron, M.G. (1983) Annu. Rev. Biochem. 52, 159–186.
2 Stiles, G.L. and Lefkowitz, R.J. (1984) Annu. Rev. Med. 35, 149–164.
3 Sperelakis, N. (1984) Am. Heart J. 107, 347–357.
4 Presti, C.F., Jones, L.R. and Lindemann, J.P. (1985) J. Biol. Chem. 260, 3860–3867.
5 Stiles, G.L., Caron, M.G. and Lefkowitz, R.J. (1984) Physiol. Rev. 64, 661–743.
6 Birnbaumer, L., Codina, J., Mattera, R., Cerione, R.A., Hildebrandt, J.D., Sunyer, T., Rojas, F.J., Caron, M.G., Lefkowitz, R.J. and Iyengar, R. (1985) Recent Prog. Hormone Res. 41, 41–99.
7 Salesse, R. and Garnier, J. (1984) Mol. Cell. Biochem. 60, 17–31.
8 Loh, H.H. and Law, P.Y. (1980) Annu. Rev. Pharmacol. Toxicol. 20, 201–234.
9 Stubbs, C.D. and Smith, A.D. (1984) Biochim. Biophys. Acta 779, 89–137.
10 Neelands, P.J. and Clandinin, M.T. (1983) Biochem. J. 212, 573–583.
11 Morson, L.A. and Clandinin, M.T. (1985) Nutr. Res. 5, 1113–1120.
12 Needham, L., Finnegan, I. and Houslay, M.D. (1985) FEBS Lett. 183, 81–86.
13 Lurie, K.G., Chin, J.H. and Hoffman, B.B. (1985) Am. J. Physiol. 249, H380–H385.
14 McMurchie, E.J., Patten, G.S., Charnock, J.S. and McLennan, P.L. (1987) Biochim. Biophys. Acta 898, 137–153.
15 Wince, L.C. and Rutledge, C.O. (1981) J. Pharmacol. Exp. Therap. 219, 625–631.
16 McMurchie, E.J., Abeywardena, M.Y., Charnock, J.S. and Gibson, R.A. (1983) Biochim. Biophys. Acta 760, 13–24.
17 McMurchie, E.J., Abeywardena, M.Y., Charnock, J.S. and Gibson, R.A. (1983) Biochim. Biophys. Acta 734, 114–124.
18 Charnock, J.S., Dryden, W.F., McMurchie, E.J., Abeywardena, M.Y. and Russell, G.R. (1983) Comp. Biochem. Physiol. 75B, 47–52.
19 Charnock, J.S., Abeywardena, M.Y., McMurchie, E.J. and Russell, G.R. (1984) Lipids 19, 206–213.
20 Gibson, R.A., McMurchie, E.J., Charnock, J.S. and Kneebone, G.M. (1984) Lipids 19, 942–951.
21 McMurchie, E.J., Gibson, R.A., Charnock, J.S. and McIntosh, G.H. (1984) Comp. Biochem. Physiol. 78B, 817–826.
22 Charnock, J.S., McIntosh, G.H., Abeywardena, M.Y. and Russell, G.R. (1985) Ann. Nutr. Metab. 29, 83–94.
23 Charnock, J.S., McLennan, P.L., Abeywardena, M.Y. and Russell, G.R. (1985) Ann. Nutr. Metab. 29, 279–288.
24 McMurchie, E.J., Gibson, R.A., Charnock, J.S. and McIntosh, G.H. (1986) Lipids 21, 315–323.
25 Charnock, J.S., McLennan, P.L., Abeywardena, M.Y. and Dryden, W.F. (1985) Ann. Nutr. Metab. 29, 306–318.
26 McLennan, P.L., Abeywardena, M.Y., Charnock, J.S. and McMurchie, E.J. (1987) J. Cardiovas. Pharmacol. 10, 293–300.
27 McLennan, P.L., Abeywardena, M.Y. and Charnock, J.S. (1985) Can. J. Physiol. Pharmacol. 63, 1411–1417.
28 Abeywardena, M.Y., McMurchie, E.J., Russell, G.R., Sawyer, W.H. and Charnock, J.S. (1984) Biochim. Biophys. Acta 776, 48–59.
29 Riemersma, R.A., Wood, D.A., Butler, S., Elton, R.A., Oliver, M., Salo, M., Nikkari, T., Vartiainen, E., Puska, P., Gey, F., Rubba, P., Maneini, M. and Fidanza, F. (1986) Br. Med. J. 292, 1423–1427.
30 McIntosh, G.H. and Looker, J.W. (1982) Lab. Anim. Sci. 32, 677–679.
31 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R. (1951) J. Biol. Chem. 193, 265–275.
32 Bowyer, D.E. and King, J.P. (1977) J. Chromatogr. 143, 473–490.
33 McMurchie, E.J., Patten, G.S., McLennan, P.L. and Charnock, J.S. (1987) Comp. Biochem. Physiol. 88B, 989–998.
34 Hanski, E., Rimon, G. and Levitzki, A. (1979) Biochemistry 18, 846–853.
35 Helmreich, E.J.M. and Elson, E.L. (1984) in Advances in Cyclic Nucleotide and Protein Phosphorylation Research, Vol. 18 (Greengard, P. and Robison, G.A., eds.), pp. 1–61, Raven Press, New York.
36 Engelhard, V.H., Esko, J.D., Storm, D.R. and Glaser, M. (1976) Proc. Natl. Acad. Sci. USA 73, 4482–4486.
37 Engelhard, V.H., Glaser, M. and Storm, D.R. (1978) Biochemistry 17, 3191–3200.
38 Whetton, A.D., Gordon, L.M. and Houslay, M.D. (1983) Biochem. J. 212, 331–338.

39 Scarpace, P.J., O'Connor, S.W. and Abrass, I.B. (1985) Biochim. Biophys. Acta 845, 520–525.
40 Levitzki, A. (1985) Biochim. Biophys. Acta 822, 127–153.
41 Limbird, L.E. and Lefkowitz, R.J. (1976) Mol. Pharmacol. 12, 559–567.
42 Needham, L. and Houslay, M.D. (1982) Biochem. J. 206, 89–95.
43 Sinensky, M., Minneman, K.P. and Molinoff, P.B. (1979) J. Biol. Chem. 254, 9135–9141.
44 Houslay, M.D. (1985) Proc. Nutr. Soc. 44, 157–165.
45 Yeagle, P.L. (1985) Biochim. Biophys. Acta 822, 267–287.
46 Tahin, Q.S., Blum, M. and Carafoli, E. (1981) Eur. J. Biochem. 121, 5–13.
47 Shekelle, R.B., MacMillan Shryock, A., Oglesby, P., Lepper, M., Stamler, J., Liu, S. and Raynor, W.J. (1981) N. Engl. J. Med. 304, 65–70.
48 Kushi, L.H., Lew, R.A., Stare, F.J., Ellison, C.R. Lozy, M., Bourke, G., Daly, L., Graham, I., Hickey, N., Mulcahy, R. and Kevaney, J. (1985) N. Engl. J. Med. 312, 811–818.
49 Hjermann, I., Velve Byre, K., Holme, I. and Leren, P. (1981) Lancet ii, 1303–1310.