Biochimica et Biophysica Acta, 942 (1988) 324–332
Elsevier

BBA 74060

Dietary cholesterol influences cardiac β-adrenergic receptor adenylate cyclase activity in the marmoset monkey by changes in membrane cholesterol status

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(Received 26 November 1987)
(Revised manuscript received 29 March 1988)

Key words: Dietary lipid; Lipid supplementation; Cholesterol; Catecholamine; Membrane lipid; Adenylate cyclase; Adrenergic receptor; (Marmoset monkey heart)

The activity of the β-adrenergic receptor/adenylate cyclase system of the marmoset monkey heart was investigated following dietary cholesterol supplementation (0.5%). After 22 weeks, plasma cholesterol levels in the cholesterol group were more than twice that of the control group. In the cholesterol-fed group, the affinity for ICYP binding to cardiac membranes was elevated more than 2-fold, while the receptor number was decreased by 31%. Isoproterenol, norepinephrine and sodium fluoride stimulated adenylate cyclase activity was significantly higher in the cholesterol-fed group although the fold stimulation over basal levels was not affected. The most prominent change in the cardiac membrane lipids was an increase in the cholesterol to phospholipid ratio in marmoset monkeys fed cholesterol. These results indicate that in the marmoset, membrane cholesterol is an important factor in determining various properties of the cardiac β-adrenergic receptor particularly receptor affinity which may impact on the response of the β-adrenergic receptor/adenylate cyclase system of the heart to catecholamines. This result is in agreement with dietary fatty acid supplements designed to increase cardiac membrane cholesterol in this animal species (McMurchie, E.J. et al. (1988) Biochim. Biophys. Acta 937, 347–358). Elevated membrane cholesterol enhances β-adrenergic receptor affinity and certain aspects of adenylate cyclase activity. This is a likely mechanism whereby atherogenic diets could promote cardiac arrhythmia in non-human primates and indeed in man.

Introduction

It is now well established that the stimulatory effects of sympathetic input to the heart are in part mediated via a GTP-dependent, β-adrenergic agonist-mediated increase in cyclic AMP levels [1] and the resulting augmentation of Ca^{2+} movement in the heart [2,3]. The β-adrenergic receptor/adenylate cyclase system and indeed hormone-sensitive adenylate cyclases in general, have been shown to be sensitive to the physical properties of their host membrane environment [4,5]. Although an increase in membrane lipid order as a result of increased membrane cholesterol has been shown to stimulate adenylate cyclase activity [6], hormone-sensitive adenylate cyclase activity has also been shown to be influenced in an opposite manner [7], or to even show a biphasic activity profile with respect to the membrane cholesterol to phospholipid ratio in liver plasma membranes upon glucagon stimulation [5]. Changes in the cardiac membrane cholesterol to phospholipid ratio have been reported to influence catecholamine-stimulated adenylate cyclase activity in the

Abbreviation: ICYP, (−)-iodocyanopindolol.

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heart following dietary lipid supplementation in rats [8] and marmoset monkeys [9].

A relationship between the nature of the dietary lipid intake and the incidence of cardiac arrhythmias has recently been reported in various animal models [10–12], and a similar relationship may exist in man. The above effects of dietary lipids on cardiac dysfunction may be based on changes in cardiac membrane lipid composition and subsequent effects on the functioning of various membrane-associated enzyme systems which are independent of atherosclerosis [8,13,14]. Prominent among these is the membrane-associated β-adrenergic receptor/adenylate cyclase system controlling the chronotropic and inotropic response of the mammalian heart to increased sympathetic drive. Overstimulation of this system may be involved in the generation of certain arrhythmias [15].

Because of the known relationship between membrane lipid composition and adenylate cyclase activity [5] and the role of this transmembrane signalling system in arrhythmogenesis, we have investigated the role of altered membrane cholesterol (induced by dietary cholesterol supplementation) on the catecholamine-sensitive adenylate cyclase system of the heart of a non-human primate animal model, using the marmoset monkey.

Materials and Methods

Adolescent male cotton-eared marmosets (*Callithrix jacchus jacchus*) approximately 12 months of age at the start of the experiment were maintained as previously described [14]. One group of five marmosets was fed a standard commercial diet consisting of a 1 : 1 mixture of Arnott Harper's (Ltd., Adelaide, Australia) greyhound chow and Milling Industries (Ltd., Adelaide, Australia) primate meal. The overall composition of this diet has previously been described [16], and on analysis contained 4.5% (w/w) total fat (Control diet). A second group of six marmosets was fed the above diet supplemented at the time of repelleting with 0.5% (w/w) cholesterol (Ajax Chemical Co., Sydney, Australia). Average body weights for the groups were 290 g (control) and 325 g (cholesterol-fed) at the start of the experiment, and 320 g (control) and 343 g (cholesterol-fed) at the end of the experiment after 22 weeks. Differences between the body weights of the two groups at the start and finish of the experiment were not significant.

Preparation of cardiac membrane fractions. Animals were killed under anaesthetic ether and the heart removed. Ventricular tissue from each marmoset heart was chopped and rinsed in ice-cold isolating medium containing 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, GmbH, Switzerland) at setting 4 for three bursts each of 30 s. A P0–500*g*, low-speed membrane fraction and a P6000*g*–46 000*g* post-mitochondrial membrane fraction (high-speed pellet), were isolated by differential centrifugation as previously described [8,17].

Adenylate cyclase assay. Adenylate cyclase (ATP pyrophosphate-lyase (cyclizing, EC 4.6.1.1) activity was measured in medium containing 50 mM Tris, 5 mM $MgCl_2$, 1 mM cAMP, 1 mM EDTA, 0.5 mM EGTA, 1 mM isobutylmethylxanthine, 1 mM dithiothreitol, 0.1% (w/v) delipidated bovine serum albumin, 10 mM creatine phosphate all adjusted to pH 7.4 with HCl. Assays were performed in triplicate at 37 °C for 20 min in a final volume of 60 μl containing 12 μg creatine phosphokinase, 100 μM GTP, 0.2 mM ATP containing between $1.5 \cdot 10^6$ and $3.0 \cdot 10^6$ cpm [α-^{32}P] ATP and appropriate additions (e.g. NaF, adrenergic agonists etc.) where indicated. Assays were initiated by the addition of 40 μg of the membrane fraction (P0–500*g*) and terminated by the addition of a stopping buffer [8,17] containing [^{3}H]cAMP as recovery marker (20 000 cpm), and processed as described previously [8,17]. This cardiac membrane fraction has previously been shown to exhibit the highest catecholamine-stimulated adenylate cyclase activity and fold stimulation by catecholamines [17]. The assay was linear over at least 20 min with a membrane protein concentration of up to 100 μg/assay.

β-Adrenergic receptor binding assay. Cardiac β-adrenergic receptor binding activity was determined using the P6000*g*–46 000*g* membrane fraction and the β-adrenergic receptor ligand,

(−)-[^{125}I]iodocyanopindolol ([^{125}I]ICYP) with specific binding being determined in the presence of 10^{-5}M propranolol using 10 points over a radioligand concentration range of 50–500 pmoles, as previously described [8,17]; specific binding was between 76% to 92%. Saturation binding assays were performed in 50 mM Tris, 10 mM $MgCl_2$, 1 mM ascorbic acid (pH 7.4) in triplicate for 60 min at 37 °C, and were initiated by the addition of ICYP. Binding assays used between 20 μg and 30 μg membrane protein in a final volume of 200 μl. This cardiac membrane fraction has previously been shown to exhibit the greatest number of β-adrenergic receptor sites and the highest per cent specific binding, with binding being linear in the range of 20–60 μg membrane protein for the marmoset monkey [17].

Protein determination. Values for the membrane protein content of cardiac membrane preparations were determined by the method of Lowry et al. [18], after solubilization of the membranes in 0.1 M NaOH and 1%(w/v) sodium dodecyl sulphate, and using bovine serum albumin as standard.

Cardiac membrane fatty acid analysis. Fatty acid analysis of total phospholipids was performed on both the P0–500*g* and the P6000*g* to 46 000*g* membrane fractions by methods previously described [8,13,14]. Separation, identification and quantification of the major phospholipid classes and the determination of the fatty acid profile of the major phospholipid classes was performed using the P0–500*g* membrane fraction by methods previously described [19,20].

Cardiac membrane phospholipid and cholesterol determination. The cholesterol to phospholipid (mol/mol) ratio was determined on total lipid extracts of the P6000*g*–46 000*g* cardiac membrane fraction. Membrane total lipids were extracted as described above for fatty acid analysis and the cardiac membrane phospholipid to cholesterol ratio determined as previously described [8].

Plasma cholesterol determination. Plasma cholesterol levels were determined after 7-weeks dietary cholesterol supplementation and at the termination of the experiment after 22 weeks. At 7 weeks, 2 ml of blood was removed from the femoral artery of restrained unanaesthetized marmosets. Plasma cholesterol levels were determined as previously described [21].

Chemicals. (−)-Isoproterenol; (±-propranolol; (−)-epinephrine; (−)-arterenol; ATP, disodium; 3-isobutyl-1-methylxanthine; creatine phosphate, disodium; creatine phosphokinase, rabbit muscle, and bovine serum albumin, fraction V were supplied by Sigma. Cyclic AMP, free acid; dithiothreitol, and GTP, dilithium, were supplied by Boehringer Mannheim. Forskolin was from Calbiochem. (−)-[^{125}I]Iodocyanopindolol, 2200 Ci/mmol, > 99% pure; [α-^{32}P]ATP, tetra (triethylammonium) salt, 3000 Ci/mmol, > 99% pure; [2,8-^{3}H]cAMP, ammonium salt, 31.1 Ci/mmol, > 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

Plasma cholesterol levels of marmoset monkeys were significantly elevated after 7 and 22 weeks dietary supplementation with 0.5% cholesterol (Table I). Although considerable variation in plasma cholesterol levels was evident at both times, the value of the cholesterol-supplemented group at 7 weeks was significantly higher than that observed in the same group after 22 weeks.

Analysis of the phospholipid and cholesterol content of marmoset cardiac P6000*g*–46 000*g* membranes isolated after 22 weeks dietary cholesterol supplementation showed that the cholesterol to phospholipid ratio was significantly elevated ($P < 0.025$) in the cholesterol-supplemented group (Table I). This was brought about by an increase in cardiac membrane cholesterol content despite a concomitant increase in the membrane phospholipid content in the cholesterol-supplemented group. Changes in the membrane lipid to protein ratio as may have occurred in this instance, have previously been reported in rat heart membranes following dietary cholesterol supplementation [8].

Fatty acid analysis of the membrane total phospholipids was performed on both cardiac membrane preparations used for biochemical measurements, i.e. the P6000*g*–46 000*g* fraction (β-adrenergic receptor binding) and the P0–500*g* fraction (catecholamine-stimulated adenylate cyclase activ-

TABLE I

EFFECT OF DIETARY CHOLESTEROL SUPPLEMENTATION ON PLASMA CHOLESTEROL LEVEL AND CARDIAC MEMBRANE LIPID CONTENT IN MARMOSET MONKEYS

Data are presented as the mean ± S.E. for $n = 5$ control (CONT) and $n = 6$ dietary cholesterol-supplemented (CHOL) marmosets with the range of values for the plasma cholesterols shown in brackets. Food was withheld for 18 h prior to taking blood samples for cholesterol determination. Cardiac membrane lipid content was determined on the P6000g–46000g marmoset heart fraction isolated after 22 weeks of dietary supplementation. The significance of differences between groups was determined by Student's t-test. [1] Difference in plasma cholesterols between dietary groups were significant ($P < 0.01$) at both 7 and 22 weeks. [2] The differences in the mean values for the cholesterol-supplemented group were significant ($P < 0.05$) when compared at 7 and 22 weeks. [3] The difference between the cholesterol to phospholipid ratios of the control and cholesterol-supplemented marmoset monkey cardiac membranes were also significant ($P < 0.025$).

Diet duration	Plasma cholesterol (mg/100 ml)	
	CONT	CHOL
7 weeks	182 ± 14 (150–235)	997 ± 209 [1,2] (684–1999)
22 weeks	182 ± 14 (156–234)	479 ± 79 [1] (291–822)
Parameter	**Membrane lipid content**	
	CONT	CHOL
nmol cholesterol/mg protein (C)	102 ± 9.0	127 ± 9.1
nmol phospholipid/mg protein (PL)	478 ± 39	514 ± 33
Cholesterol to phospholipid (C/PL)	0.213 ± 0.005	0.247 ± 0.011 [3]

ity). Some differences were evident in the fatty acid composition between the two different membrane fractions when comparing similar dietary groups (Table II). However following dietary cholesterol supplementation, no major differences were evident when comparing within each cardiac membrane fraction except for that of arachidonic acid and the total ($n - 6$) polyunsaturates in the P6000g–46000g membrane fraction, as noted in the legend to Table II.

Analysis of the phospholipid class distribution and the effect of dietary cholesterol supplementation on the fatty acid distribution of the major membrane phospholipid species could only be performed on the low-speed P0–500g membrane fraction because of the limited amount of membrane material available from the heart of this particular animal species. The effect of dietary cholesterol on the distribution of the major phospholipid classes in marmoset cardiac P0–500g membranes is shown in Table III. Dietary cholesterol treatment decreased significantly the relative proportions of cardiolipin (DPG) and the combined phosphatidylserine, phosphatidylinosi-

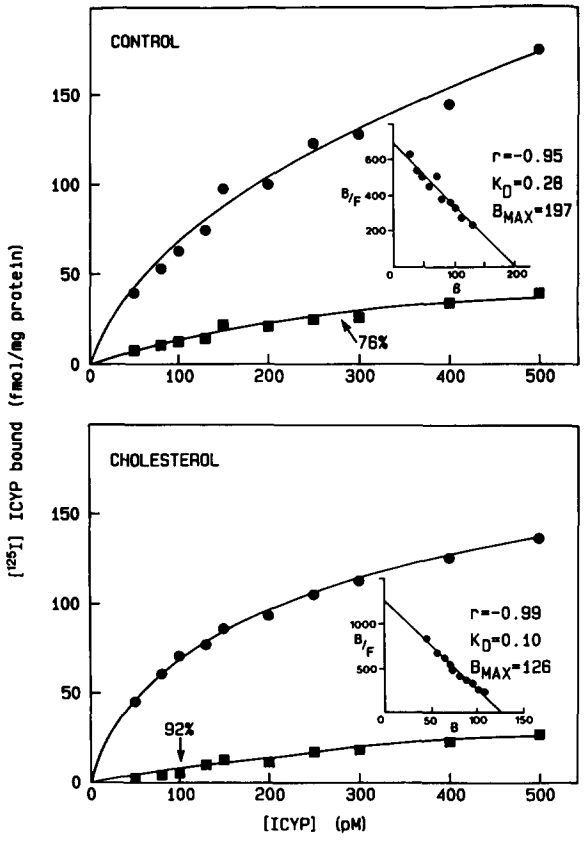

Fig. 1. Saturation binding curves for ICYP with marmoset monkey cardiac membranes. Curves were generated by first normalizing for protein and then averaging data from $n = 4$ control animals and $n = 5$ cholesterol-supplemented animals. Aliquots of the high speed membrane pellet (P6000g–46000g) were incubated in the presence (■) or absence (●) of 10^{-5} M propranolol at 10 concentrations of the radioligand ICYP in the range of 50–500 pmol at 37 °C for 60 minutes. Specific binding is plotted in the Scatchard form (inset) showing values for the respective correlation coefficient (r), dissociation constant (K_d) and receptor number (B_{max}). The percentage of specific binding at K_d is indicated by the arrows.

TABLE II

TOTAL PHOSPHOLIPID FATTY ACID COMPOSITION OF MARMOSET HEART VENTRICLE P6000g–46000g AND P0–500g MEMBRANES FOLLOWING DIETARY CHOLESTEROL SUPPLEMENTATION

Major fatty acids are designated by the number of carbon atoms followed by the number of double bonds and the particular unsaturated fatty acid series ($n-x$) as defined in Ref. 9. Data are shown as the mean ± S.E. Differences between the means were only significant for 20:4($n-6$) ($P < 0.001$) and the total ($n-6$) polyunsaturated fatty acids ($P < 0.01$) in the P6000g–46000g group. U.I. is the unsaturation index as defined in Ref. 13. CONT, marmoset fed control diet; CHOL, dietary cholesterol-supplemented marmoset.

Major fatty acid	P6000g–46000g		P0–500g	
	CONT ($n=4$)	CHOL ($n=6$)	CONT ($n=5$)	CHOL ($n=6$)
16:0	12.0±0.4	12.7±0.5	11.9±0.2	11.3±0.2
16:1 ($n-7$)	0.8±0.1	1.0±0.1	0.8±0.1	0.8±0.1
18:0	17.6±0.4	16.5±0.7	17.3±0.3	17.7±0.2
18:1($n-9$)	13.0±0.2	14.3±1.0	13.7±0.4	13.7±0.3
18:2($n-6$)	20.3±0.9	18.2±0.9	25.3±0.6	26.1±0.3
20:3($n-6$)	2.0±0.1	1.7±0.2	1.0±0.1	1.1±0.1
20:4($n-6$)	16.6±0.4	13.3±0.4	18.3±0.3	18.5±0.3
20:5($n-3$)	1.0±0.3	1.8±0.3	0.8±0.1	0.8±0.1
22:4($n-6$)	1.0±0.1	1.1±0.3	0.6±0.1	0.5±0.1
24:0	2.0±0.1	3.2±0.8	2.2±0.5	1.8±0.1
22:5($n-3$)	3.8±0.4	2.9±0.3	3.0±0.1	2.7±0.1
22:6($n-3$)	5.3±0.4	5.6±0.8	4.9±0.3	5.0±0.2
Satd.(S)	35.4±0.4	35.5±0.8	31.4±0.5	30.8±0.3
Unsatd.	65.5±0.4	64.5±0.8	68.6±0.5	69.2±0.3
Polyunsatd.(P)	50.5±0.5	48.9±0.9	54.0±0.2	54.7±0.3
P/S	1.46	1.38	1.72	1.78
Total($n-6$)	39.1±1.1	34.9±0.6	45.3±0.4	46.2±0.4
Total($n-3$)	10.7±0.8	10.8±0.9	8.8±0.4	8.5±0.2
(n-6)/($n-3$)	3.6	3.2	5.1	5.4
U.I.	185	173	192	194

TABLE III

THE EFFECT OF A CHOLESTEROL-SUPPLEMENTED DIET ON THE DISTRIBUTION OF MAJOR PHOSPHOLIPID CLASSES IN MARMOSET HEART P0–500g MEMBRANES

Results are the percent distribution (mean ± S.E.) of the major phospholipid classes of the total phospholipids of the P0–500g membrane fraction. PC, phosphatidylcholine; PE, phosphatidylethanolamine; DPG, diphosphatidylglycerol (cardiolipin). 'Others' represent the minor components comprising phosphatidylserine, phosphatidylinositol and sphingomyelin. Significant differences are indicated by [1] $P < 0.005$ and [2] $P < 0.02$. CONT, marmoset fed control diet; CHOL, dietary cholesterol-supplemented marmoset.

Major phospholipid	CONT ($n=5$)	CHOL ($n=6$)
PC	50.2±1.0	53.9±1.9
PE	32.9±1.7	33.7±1.3
DPG	10.6±0.5	7.7±0.4 [1]
'Others'	6.3±0.4	4.7±0.4 [2]

tol and sphingomyelin fraction. The relative proportions of phosphatidylcholine and phosphatidylethanolamine were unaffected.

The effects of dietary cholesterol on the fatty acid composition of the major phospholipid classes of the P0–500g cardiac membrane fraction are shown in Table IV. Each of these three phospholipids exhibited distinct fatty acid profiles with regard to a number of fatty acids, e.g. palmitic (16:0), stearic (18:0), linoleic (18:2 ($n-6$)) and arachidonic (20:4 ($n-6$)), which has been noted previously when examining lipids extracted from marmoset ventricular homogenate material [20]. Dietary cholesterol supplementation did not have a profound effect on the fatty acid profile of any of these major phospholipid classes, except where noted in the legend to Table IV. It must be pointed out that fatty acid analysis was not performed on the minor phospholipid species, phos-

TABLE IV

FATTY ACID COMPOSITION OF THE MAJOR PHOSPHOLIPID CLASSES FROM MARMOSET HEART P0–500g MEMBRANES FROM ANIMALS FED CONTROL OR CHOLESTEROL-SUPPLEMENTED DIETS

Data shown are the mean ± S.E. Significant differences (* $P < 0.05$) were determined only between measurements within the one phospholipid class. CONT, marmoset fed control diet; CHOL, dietary cholesterol-supplemented marmoset.

Major fatty acid	Major phospholipid class					
	PC		PE		DPG	
	CONT ($n = 5$)	CHOL ($n = 6$)	CONT ($n = 5$)	CHOL ($n = 5$)	CONT ($n = 5$)	CHOL ($n = 4$)
16:0	25.4 ± 0.4	26.2 ± 2.2	3.6 ± 0.4	3.1 ± 0.5	3.8 ± 0.4	2.8 ± 0.2
16:1($n-7$)	0.6 ± 0.3	0.6 ± 0.4	0.5 ± 0.2	0.9 ± 0.3	1.0 ± 0.1	0.9 ± 0.1
18:0	12.8 ± 0.3	13.4 ± 0.8	29.6 ± 0.5	29.1 ± 1.2	6.1 ± 0.8	3.5 ± 0.5 *
18:1($n-9$)	16.6 ± 0.7	19.7 ± 1.5	8.7 ± 0.9	10.2 ± 1.9	14.1 ± 1.2	17.6 ± 4.6
18:2($n-6$)	21.2 ± 0.7	20.1 ± 1.1	6.3 ± 0.4	6.0 ± 0.2	59.8 ± 2.8	66.2 ± 4.2
20:3($n-6$)	1.3 ± 0.1	1.8 ± 0.2	0.9 ± 0.1	1.3 ± 0.4	0.9 ± 0.1	0.9 ± 0.1
20:4($n-6$)	14.3 ± 0.9	11.0 ± 0.9 *	31.9 ± 0.6	31.3 ± 1.0	6.7 ± 0.9	3.6 ± 0.5
20:5($n-3$)	0.9 ± 0.1	0.6 ± 0.1	1.3 ± 0.2	0.9 ± 1.0	0.3 ± 0.1	0.3 ± 0.1
22:4($n-6$)	0.5 ± 0.1	0.7 ± 0.2	1.2 ± 0.1	1.4 ± 0.3	1.0 ± 0.2	0.3 ± 0.2
24:0	1.3 ± 0.1	1.4 ± 0.1	3.5 ± 0.2	3.8 ± 0.6	2.3 ± 0.5	1.9 ± 0.2
22:5($n-3$)	2.2 ± 0.1	2.0 ± 0.2	4.7 ± 0.1	3.8 ± 0.3 *	1.7 ± 0.3	1.0 ± 0.2
22:6($n-3$)	3.1 ± 0.2	2.6 ± 0.4	8.0 ± 0.2	8.3 ± 1.1	2.3 ± 0.8	1.1 ± 0.1
Satd.(S)	39.5 ± 0.3	41.0 ± 2.7	36.6 ± 0.3	36.0 ± 1.1	12.1 ± 1.4	8.2 ± 0.8
Unsatd.	61.5 ± 0.3	59.0 ± 2.7	63.4 ± 0.3	64.0 ± 1.1	87.9 ± 1.4	91.8 ± 0.8
Polyunsatd.(P)	44.8 ± 0.8	38.7 ± 2.3 *	54.3 ± 0.4	52.9 ± 1.4	72.9 ± 1.0	72.7 ± 4.4
P/S	1.1	0.9	1.5	1.5	6.0	8.9
Total($n-6$)	37.2 ± 0.7	33.5 ± 1.7	40.3 ± 0.3	40.0 ± 0.5	68.5 ± 1.8	70.9 ± 4.5
Total($n-3$)	6.2 ± 0.4	5.2 ± 0.7	14.0 ± 0.3	13.0 ± 1.3	4.4 ± 1.1	2.4 ± 0.2
($n-6$)/($n-3$)	6.0	6.4	2.9	3.1	15.6	29.5
U.I.	156	141	235	229	193	182

phatidylserine, phosphatidylinositol and sphingomyelin. Therefore, we cannot rule out the possibility that dietary-induced changes occurring in these minor phospholipids may have had quite dramatic effects on cardiac membrane function.

Changes in cardiac β-adrenergic receptor affinity and number were apparent after 22 weeks dietary cholesterol supplementation (Fig. 1). The affinity for ICYP binding was increased significantly following dietary cholesterol supplementation; K_d value reduced from 0.254 ± 0.03 nM (mean ± S.E., $n = 4$, derived from the average of the individual binding experiments and Scatchard determinations) in the control group, to 0.106 ± 0.014 nM ($n = 5$) in the cholesterol-supplemented group. Accompanying this increase in receptor affinity, cardiac beta-adrenergic receptor number was lowered from 185 ± 16 fmol/mg protein in the control group to 128 ± 16 fmol/mg protein in the cholesterol-supplemented group. The signifi-

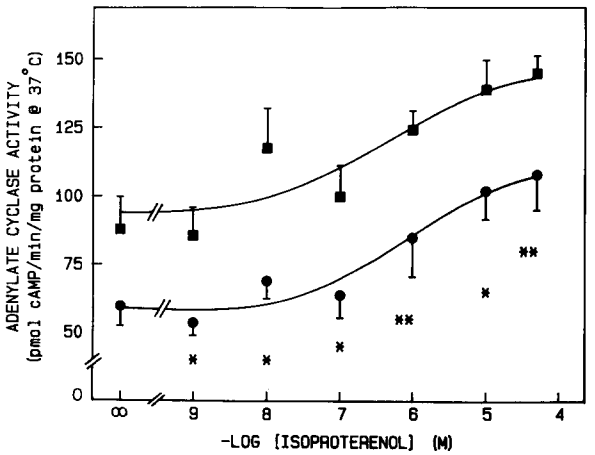

Fig. 2. Effect of dietary cholesterol supplementation on marmoset cardiac isoproterenol-stimulated adenylate cyclase activity using the P0–500g membrane fraction. Data are shown as the mean ± S.E. for $n = 4$ control (●) and $n = 6$ cholesterol-supplemented (■) animals. Differences between means were significant at $P < 0.05$ (*) and $P < 0.025$ (**) as determined by Student's t-test.

TABLE V

EFFECT OF DIETARY CHOLESTEROL SUPPLEMENTATION ON MARMOSET HEART ADENYLATE CYCLASE ACTIVITY

Adenylate cyclase activity expressed as pmol cAMP/min per mg protein at 37 °C is presented as the mean ± S.E. for $n = 4$ animals in the control (CONT) and $n = 6$ animals in the dietary cholesterol group (CHOL). The significance of differences between means was determined by Student's t-test with [1] $P < 0.05$, [2] $P < 0.025$ and [3] $P < 0.01$. Δ cAMP is the maximum change in cAMP across the isoproterenol dose curve. * Fold stimulation is that rate compared over basal.

Addition	CONT		CHOL	
	rate	fold *	rate	fold *
Basal	59.6–7.3	–	87.8 ± 12.0	–
Isoproterenol (10^{-9} M)	53.5 ± 4.7	–	85.6 ± 10.3	–
Isoproterenol ($5 \cdot 10^{-5}$ M)	108 ± 13.0	1.81	145 ± 6.5 [2]	1.65
Isoproterenol (10^{-5} M) plus propranolol (10^{-4} M)	63.6 ± 8.0	1.07	92.9 ± 10.0	1.06
Δ cAMP	56.4 ± 7.9	–	66.7 ± 14.1	–
Epinephrine ($5 \cdot 10^{-5}$ M)	97.1 ± 16.0	1.63	136 ± 11	1.55
Norepinephrine ($5 \cdot 10^{-5}$ M)	90.9 ± 10.4	1.52	124 ± 8.6 [1]	1.41
NaF (10 mM)	184 ± 16.7	3.09	265 ± 15.0 [3]	3.02
Forskolin (100 μM)	646 ± 31	10.84	721 ± 20.0	8.21
Propranolol (10^{-4} M)	74.7 ± 10.1	1.25	99.0 ± 11.0	1.13

cance of differences between the means was determined by Student's t-test and was $P < 0.005$ for K_d and $P < 0.05$ for B_{max}. The correlation coefficient (r) for the individual Scatchard plots was always between −0.95 and −0.99. The plots in Fig. 1 are not representative curves from single experiments, but are derived from the number of binding experiments indicated in the figure legend, normalized for protein, and then averaged to generate the binding curves and Scatchard plots shown. The specific binding at K_d was 76% for the control group and 92% for the cholesterol-supplemented group.

Basal and (total) isoproterenol-stimulated adenylate cyclase activity associated with cardiac membranes was significantly increased in marmosets supplemented with dietary cholesterol for all isoproterenol concentrations tested (10^{-9} M to $5 \cdot 10^{-5}$ M) compared to the control group (Fig. 2). ED_{50} values for isoproterenol stimulation of adenylate cyclase activity were not influenced by dietary cholesterol and remained at about $8 \cdot 10^{-7}$ M, as previously reported [17]. Other parameters associated with the activation of cardiac adenylate cyclase activity were also increased following dietary cholesterol supplementation (Table V). Although the increases in adenylate cyclase activity were only statistically significant for norepinephrine ($P < 0.05$) and NaF ($P < 0.01$), all other parameters associated with the activation of adenylate cyclase activity as shown in Table V, were increased in the cholesterol-supplemented group. While the absolute rates of adenylate cyclase activity following agonist or activator stimulation were higher following dietary cholesterol supplementation, the fold stimulation was the same or even slightly less in this dietary group (Table V). Indeed, the elevation of catecholamine-stimulated adenylate cyclase activity is mainly accountable by the basal activity being enhanced by the cholesterol-supplemented diet. The stimulation of marmoset cardiac adenylate cyclase activity by propranolol alone has previously been reported [17].

Discussion

Dietary cholesterol had a significant effect on the β-adrenergic receptor/adenylate cyclase system of the marmoset heart with changes in receptor properties being the most prominent. Elevated plasma cholesterol levels resulting from cholesterol supplementation increased the cardiac membrane cholesterol to phospholipid ratio and β-adrenergic receptor affinity, as well as elevating the absolute rates of isoproterenol, norepinephrine and NaF stimulated adenylate cyclase activity, but did not

alter the fold stimulation. Changes in the membrane phospholipid fatty acid profile and the phospholipid class distribution, together with the fatty acid profiles of the major phospholipids were not dramatic and therefore probably not major factors influencing the changes observed in the activity of the β-adrenergic receptor/adenylate cyclase system. However, the contribution of dietary cholesterol-induced changes in some or all of the minor membrane phospholipid components, (phosphatidylserine, phosphatidylinositol and sphingomyelin), cannot be discounted from the results of the present study. Concomitant with the changes in catecholamine-stimulated adenylate cyclase activity, β-adrenergic receptor number was decreased possibly indicating receptor down-regulation in response to elevated adenylate cyclase activity. This relationship has been noted previously where catecholamine-stimulated adenylate cyclase activity has been increased by various dietary lipid treatments [8,9].

The changes in catecholamine-stimulated adenylate cyclase activity reported in this study are consistent with other reports that this membrane-associated signalling system is sensitive to the membrane lipid composition, particularly when membrane physico-chemical properties may be altered [4,5]. Dietary saturated fatty acid supplementation in the form of sheep kidney fat significantly increases catecholamine-stimulated adenylate cyclase activity in marmoset monkey cardiac membranes in combination with an increase in the membrane cholesterol to phospholipid ratio [9]. Furthermore, such dietary lipid treatment induced changes in the β-adrenergic receptor binding parameters similar to that described in the present study [9]. In addition, an increase in the cholesterol to phospholipid ratio in heart membranes of the rat following dietary cholesterol supplementation also increased catecholamine-stimulated adenylate-cyclase activity but did not alter β-adrenergic receptor affinity [8].

The overall coupling and activity of hormone receptor, guanine nucleotide regulatory protein(s), and adenylate cyclase catalytic unit therefore appears to be influenced by alterations in host membrane cholesterol. While in some systems an increase in adenylate cyclase activity has been reported when lipid order has been increased by in vitro cholesterol incorporation [6], the opposite effect is seen with liver plasma membrane associated adenylate cyclase activity [7]. In that latter study, the fold stimulation of basal adenylate cyclase activity by glucagon, sodium fluoride and forskolin was markedly elevated for cholesterol-enriched membranes and decreased for cholesterol-depleted membranes [7]. In our study, no such dramatic change in fold stimulation was apparent upon altering membrane cholesterol status. These contradictory observations may be reconciled by several factors. Firstly, the manner by which changes in membrane cholesterol are induced in a particular experiment, differ significantly. Hence there is the possibility that long-term dietary lipid manipulations may induce adaptive changes in membrane physico-chemical properties not apparent with in vitro membrane cholesterol manipulation. Secondly, as described by Houslay [5], the activity of hormone-sensitive adenylate cyclase may show a biphasic response to changes in membrane cholesterol, although this is only cited for glucagon-stimulated adenylate cyclase activity of rat liver plasma membranes following in vitro membrane cholesterol manipulation. The ability of cholesterol to change various properties of adenylate cyclase activation via its effect on membrane physico-chemical properties may relate to whether or not the activity of the hormone-receptor complex is initially optimized with respect to the membrane cholesterol to phospholipid ratio (or more correctly) to membrane bilayer fluidity, and that such a condition remains throughout the isolation and assay of the complex. Thirdly, the effect of cholesterol in complexing inhibitory acidic phospholipids which in turn influence hormone-sensitive adenylate cyclase activity, must not be overlooked [22]. Finally, consideration must also be given to the effect changes in hormone receptor properties per se could induce to the overall functioning of the adenylate cyclase following membrane lipid manipulation. Although the manner by which cholesterol influenced β-adrenergic receptor affinity in the present study is unknown, it is clear that the dramatic increase in receptor affinity following dietary cholesterol supplementation would imply that this part of the overall transmembrane signalling complex is also sensitive to changes in membrane lipid composition, and hence

membrane physico-chemical properties.

There is an increasing recognition of the link between dietary lipids and heart disease via the process of atherosclerosis [23]. However, besides its role in atherosclerosis, cholesterol has been reported to influence various membrane-related functions which could have bearing on atherosclerosis, with the relationship between membrane cholesterol content and calcium uptake via the calcium channel, being one such example [24,25]. Sudden cardiac death is a facet of heart disease involving a disturbance to cardiac rhythm and automaticity [26] which in animal models at least, is influenced by dietary lipids [10–12]. The generation of arrhythmias and ectopic beats can occur via an enhancement of intracellular Ca^{2+} ion uptake by a cAMP-dependent phosphorylation of the slow calcium channel [2,3]. Elevated membrane cholesterol could enhance this process by its effect on cAMP production via catecholamine stimulated adenylate cyclase; an effect mediated by changes in cardiac β-adrenergic receptor affinity.

The results of this study suggest that an elevation in plasma cholesterol could significantly influence the physiology and pharmacology of the mammalian heart under conditions of increased sympathetic drive by affecting the β-adrenergic receptor/adenylate cyclase system of the heart by way of changes in the cardiac membrane cholesterol to phospholipid ratio. Indeed the results provide a biochemical link between atherogenic diets and their promoting effect on cardiac arrhythmogenesis in this [12] and other animal models [10,11]. Therefore consideration of the effects of dietary lipids on heart disease should also take into account the effects of dietary lipid perturations in membrane lipid composition. This is particularly so in terms of membrane-associated cholesterol and its effects on transmembrane signalling systems instrumental in the control of cardiac contractility and rhythmicity.

Acknowledgements

We thank Mr. P. Royle and Mr. G. Crook for care of marmosets and diet preparation and Ms. S. Crouch and Ms. J. Rinaldi for technical assistance. We thank Dr. R. Gibson for assistance with the fatty acid analysis and Dr. G. McIntosh and Ms. F. Bulman for plasma cholesterol analysis. This work was funded in part from a grant-in-aid from the National Heart Foundation of Australia (to E.J.M.)

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