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The interaction of dietary fatty acid and cholesterol on catecholamine-stimulated adenylate cyclase activity in the rat heart

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Diets supplemented with high levels of saturated or unsaturated fatty acids supplied by addition of sheep kidney fat or sunflower seed oil, respectively, were fed to rats with or without dietary cholesterol. The effects of these diets on cardiac membrane lipid composition, catecholamine-stimulated adenylate cyclase and β-adrenergic receptor activity associated with cardiac membranes, were determined. The fatty acid-supplemented diets, either with or without cholesterol, resulted in alterations in the proportion of the $(n-6)$ to $(n-3)$ series of unsaturated fatty acids, with the sunflower seed oil increasing and the sheep kidney fat decreasing this ratio, but did not by themselves significantly alter the ratio of saturated to unsaturated fatty acids. However, cholesterol supplementation resulted in a decrease in the proportion of saturated and polyunsaturated fatty acids and a dramatic increase in oleic acid in cardiac membrane phospholipids irrespective of the nature of the dietary fatty acid supplement. The cholesterol / phospholipid ratio of cardiac membrane lipids was also markedly increased with dietary cholesterol supplementation. Although relatively unaffected by the nature of the dietary fatty acid supplement, catecholamine-stimulated adenylate cyclase activity was significantly increased with dietary cholesterol supplementation and was positively correlated with the value of the membrane cholesterol / phospholipid ratio. Although the dissociation constant for the β-adrenergic receptor, determined by [^{125}I](−)-iodocyanopindolol binding, was unaffected by the nature of the dietary lipid supplement, the number of β-adrenergic receptors was dramatically reduced by dietary cholesterol and negatively correlated with the value of the membrane cholesterol / phospholipid ratio. These results indicate that the activity of the membrane-associated β-adrenergic / adenylate cyclase system of the heart can be influenced by dietary lipids particularly those altering the membrane cholesterol / phospholipid ratio and presumably membrane physico-chemical properties. In the face of these dietary-induced changes, a degree of homeostasis was apparent both with regard to membrane fatty acid composition in response to an altered membrane cholesterol / phospholipid ratio, and to down regulation of the β-adrenergic receptor in response to enhanced catecholamine-stimulated adenylate cyclase activity.

Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid: EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulphate. Diets: REF, reference, standard laboratory diet; SKF, sheep kidney fat-supplemented diet; SSO, sunflower seed oil-supplemented diet.

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Introduction

Many membrane-associated enzymes have now been shown to depend on phospholipids for their activity with changes in the physico-chemical properties of membrane lipids influencing mem-

brane enzyme activity [1–3]. Thus changes in the membrane fatty acid composition [4–7], the cholesterol to phospholipid ratio [8–11], or temperature-induced changes in the physical state of the membrane lipids [2,12], have all been shown to influence membrane enzymes in some manner.

Alterations in the lipid composition of cardiac membranes have been achieved using a wide range of dietary lipid supplements [13–16]. While dietary lipids have the capacity to alter some of the compositional characteristics of membrane lipids, in general, cell membranes display considerable homeostasis despite wide variations in dietary lipid intake [6,17,18]. Feeding diets rich in saturated fatty acids (supplied by supplementation with sheep kidney fat) or polyunsaturated fatty acids (by supplementation with sunflower seed oil), does not result in dramatic changes in the proportion of saturated or unsaturated fatty acids in the membrane phospholipids from various tissues [17,18]. This is particularly so for membranes isolated from the heart of rats and marmoset monkeys [6,17,18]. Despite a homeostatic maintenance of the level of cardiac membrane lipid saturation in these animals, significant changes are observed in the proportion of the various series of polyunsaturated fatty acids, particularly those of the $n-6$ (linoleic) and $n-3$ (linolenic) series, when feeding the above types of dietary supplements [5,12,17–21]. For the marmoset, feeding these fatty acid-supplemented diets results in changes to the cholesterol/phospholipid ratio of sarcolemmal-enriched membrane fractions of the heart [7,22]. Accompanying these changes in the membrane cholesterol/phospholipid ratio in the marmoset heart is a significantly enhanced catecholamine-stimulated adenylate cyclase activity [7,22]. These dietary fatty acid effects on the membrane cholesterol/phospholipid ratio and the activity of adenylate cyclase are not observed in the rat heart [7,22].

The above observations are significant with regard to the reported effects of dietary lipids on cardiac contractility during catecholamine stimulation [23]. At the molecular level, it is possible that these dietary lipid changes in contractility are being mediated by alteration in the activity of the membrane-associated β-adrenergic adenylate cyclase system of the heart [7,22]. This multicomponent, enzyme-receptor system mediates in part, the chronotropic and inotropic response of the heart to catecholamines released during sympathetic stimulation [24–26]. The activity of a number of hormone-sensitive adenylate cyclase systems has been shown to be influenced by dietary lipid supplementation and its resultant effect on membrane lipid composition [8,16,27–29]. In general, hormone-activated adenylate cyclases appear to be particularly sensitive to the physicochemical properties of their host membranes with the membrane cholesterol/phospholipid ratio being a major determinant in this regard [9–11, 30,31].

To integrate the above observations on dietary lipids, cardiac contractility, membrane cholesterol and hormone-sensitive adenylate cyclase, this study investigates the effect of dietary fatty acid and cholesterol supplementation on the activity of the β-adrenergic adenylate cyclase system of the rat heart. The activity of this enzyme-receptor system is investigated with regard to changes in cardiac membrane lipid composition induced by dietary lipid supplementation.

Materials and Methods

Rats

Adult male (Hooded Wistar) rats were maintained on the lipid-supplemented diets described below for a period of 13 weeks for those rats receiving a cholesterol and fatty acid supplement, or 20 weeks for those rats receiving only a fatty acid supplement. The different periods of feeding for rats on the fatty acid supplemented diets with or without cholesterol were chosen so as to provide equivalent body weight at the termination of the experiment. Rats not fed the cholesterol supplement had an initial body weight of 266 to 273 g and a final body weight of 443 to 464 g. Rats fed the cholesterol supplement had an initial body weight of 158 to 173 g and a final body weight of 444 to 464 g. After dietary lipid supplementation for the appropriate time, rats were killed by decapitation and the heart removed for immediate preparation of membrane fractions.

Rat diets

A total of six dietary regimes were used, three

with and three without added cholesterol. Three of these diets were as follows: Standard commercial laboratory chow (Milling Industries Ltd., Adelaide, South Australia) which contained 4% (w/w) total fat which was designated the reference (REF) diet. This diet was crushed and repelleted in a manner similar to the two lipid-supplemented diets described below. A second group of rats was fed the above diet supplemented at the time of repelleting with sunflower seed oil (Nuttelex Pty. Ltd., Melbourne, Victoria), to give a total of 16% (w/w) total fat (the SSO diet). A third group of rats was fed the reference diet supplemented with sheep kidney (perirenal) fat, a natural source of saturated fat. This diet contained 16% (w/w) total fat and was designated the SKF diet. The amount of cholesterol present in each of these three diets was less than 0.04% (w/w) of the diet [20]. The fatty acid composition of the dietary lipid supplements has been previously described [6,23], as has the fatty acid composition of the final fabricated diets. A further three diets were prepared as described above but were supplemented with 2% (w/w) cholesterol at the time of diet preparation. These diets were designated REF + CHOL; SKF + CHOL; SSO + CHOL. All diets were supplied ad libitum.

Preparation of cardiac membrane fractions

Ventricular tissue from rat 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 3 bursts each of 30 s. The brei was filtered through 4 layers of wide-weave cheesecloth and centrifuged at $500 \times g$ for 15 min and the supernatant saved. The pellet was resuspended in isolating medium and centrifuged as above. The resulting pellet was resuspended in isolating medium to a final concentration of about 7 mg protein/ml (P0–500 g, low-speed membrane pellet). The supernatant from this and the first centrifugation were combined and centrifuged at $6000 \times g$ for 15 min and the pellet discarded. The supernatant remaining after this step was centrifuged at $46\,000 \times g$ for 30 min. The resulting pellet was resuspended in isolating medium and centrifuged at $46\,000 \times g$ for 30 min and the pellet resuspended in 20 mM Tris/2 mM $MgCl_2$ (pH 7.4) at a concentration of 4 to 5 mg protein/ml (P6000g–46 000g post-mitochondrial membrane fraction, high-speed pellet). If not used immediately, aliquots of the above cardiac membrane preparations were immediately frozen at −80°C.

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 cyclic AMP (cAMP)/1 mM EDTA/0.5 mM EDTA/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 usually performed in triplicate were in a final volume of 60 μl containing 12 μg creatine phosphokinase, 100 μM GTP, 0.2 mM ATP containing $(1.5–3.0) \cdot 10^6$ cpm [α-^{32}P]ATP and appropriate additions (e.g. NaF, adrenergic agonists, etc.) where indicated. The reaction was initiated by the addition of 10 μl (40 μg of membrane protein) of the low speed membrane pellet (P0–500g), and was carried out for 20 min at 37°C. The reaction was terminated by the addition of 100 μl of stopping medium containing 2% (w/v) SDS, 40 mM ATP, 1.4 mM cAMP and [^{3}H]cAMP (20 000 cpm) added as a recovery marker. [^{32}P]cAMP was determined by sequential Dowex and alumina column chromatography according to Salomon [32]. Although the production of [^{32}P]cAMP was linear over 20 min and with membrane protein concentrations up to 100 μg, in subsequent assays the protein concentration was adjusted to give exactly 40 μg membrane protein.

β-Adrenergic receptor assay

Cardiac β-adrenergic receptor binding activity was determined using the P6000g–46 000g membrane fraction and the β-adrenergic radioligand (−)-[^{125}I]iodocyanopindolol (ICYP). Competition binding assays were done in 50 mM Tris/10 mM $MgCl_2$/1 mM ascorbic acid (pH 7.4 at 37°C) for 60 min. Assays were performed in duplicate in a final volume of 200 μl containing a range of ICYP (30–700 pM) concentrations, with or without

10^{-5} M (±)-propranolol to determine specific binding, and 20 μl of the cardiac membrane preparation which was adjusted to give exactly 80 μg membrane protein per assay. Assays were initiated by the addition of ICYP and were terminated by the addition of 3 ml ice-cold 50 mM Tris/10 mM $MgCl_2$ (pH 7.4) followed immediately by rapid filtration through 2.5 cm Whatman GF/C filters and thereafter by two further 3-ml washes of the above medium. Filters were dried and counted in a LKB 1272 Clinigamma counter (Lindbrook International) at 81.5% efficiency. Values for the filter blanks which were subtracted from the raw data, were determined using the appropriate concentration of ICYP with or without 10^{-5} M propranolol but with no added cardiac membrane preparation. The dissociation constant (K_d) and the receptor number (B_{max}) were determined using Scatchard plots [33] which were compiled from the specific binding data, with lines being fitted by linear-regression analysis. For binding experiments the hearts of three animals were pooled for the preparation of each high-speed (P6000*g*–46 000*g*) membrane preparation.

Protein determination

Values for the membrane protein content of the two cardiac membrane preparations used were determined by the method of Lowry et al. [34], after solubilization of the membranes in 0.1 M NaOH and 1% (w/v) SDS 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 P6000*g*–46 000*g* (high-speed membranes) fraction as previously described [5,6,18].

Cardiac membrane phospholipid and cholesterol determination

Membrane phospholipid (nmol phospholipid/mg protein) and membrane total cholesterol (nmol cholesterol/mg protein) content, and the cholesterol to phospholipid (mol/mol) ratio were determined on total lipid extracts of the P6000*g*–46 000*g* cardiac membrane fraction. Membrane total lipids were extracted as previously described [5,6,18]. Membrane phospholipid content was determined using the method of Bowyer and King [36] with egg yolk phosphatidylethanolamine as standard. Briefly, 60 μg of the total lipid extract was digested in 0.2 ml 60% (v/v) perchloric acid. 1.5 ml of the colour reagent described by Bowyer and King (1977) [36], was added to 0.2 ml aliquots of the digest and the absorbance at 660 nm determined. For membrane cholesterol determination, 0.1 ml of 10 M 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. After cooling, 2 ml of water was added and total cholesterol was extracted with three washes of 5 ml petroleum ether. Campesterol was added before hydrolysis as a recovery marker. Samples were analysed for cholesterol 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

(−)-Isoproterenol; DL-propranolol; (−)-epinephrine; (−)-arterenol; cholesterol; ATP, disodiumsalt; 3-isobutyl-1-methylxanthine; creatine phosphate, disodiumsalt; cholestane; campesterol; creatine phosphokinase, rabbit muscle; egg yolk phosphatidylethanolamine; and bovine serum albumin, fraction V, were supplied by Sigma. Cyclic AMP, free acid; dithiothreitol; GTP, dilithiumsalt, were supplied by Boehringer Mannheim. Forskolin was from Calbiochem. Cholesterol for dietary supplementation was supplied by Labchem. (−)-[^{125}I]Iodocyanopindolol (2200 Ci/mmol, > 99% pure); [α-^{32}P]ATP, tetra(triethylammonium) salt (3000 Ci/mmol, > 99% pure); [2,8-^{3}H]adenosine 3′,5′-cyclic phosphate, 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 highest reagent grade available.

Results

Membrane lipid analysis

Membrane lipid analysis was performed on the

high-speed cardiac membrane preparation that was obtained by centrifuging the post-mitochondrial supernatant at $46\,000 \times g$ (i.e. P6000g–46 000g). From other studies [37], this particular membrane fraction was found to exhibit the greatest number of β-adrenergic receptors (identified by (–)-iodocyanopindolol binding), when various membrane fractions from cardiac tissue were assayed for β-adrenergic receptor binding activity. On the basis of the relative number of β-adrenergic receptors and due to the limited amount of cardiac tissue available, this particular high-speed membrane fraction was used for both membrane lipid analysis and β-adrenergic receptor assays. The above protocol was favoured over using a fraction that may have exhibited greater sarcolemmal membrane purification, but was of insufficient yield to undertake both membrane lipid analysis and β-adrenergic receptor binding assays.

The dietary lipid treatments resulted in significant changes in the fatty acid composition of the phospholipids of rat heart membranes: Differences were evident as a result of both the nature of the fatty acid supplement and the presence of cholesterol. Feeding the sheep kidney fat diet which was relatively high in saturated fatty acids did not significantly alter the proportion of saturated or unsaturated fatty acids in rat cardiac membrane phospholipids, nor did it alter the polyunsaturated to saturated fatty acid ratio in comparison to that observed when feeding the reference diet (Table I). However, despite the failure to alter the above parameters, changes were evident in some of the individual saturated and unsaturated fatty acids. Of the major saturated fatty acids, the proportion of 16 : 0 decreased and the proportion of 18 : 0 increased relative to the reference diet. With regard to the unsaturated fatty acids, only changes in the proportion of the polyunsaturated fatty acids were significant. The net result was a relative decrease in the $(n-6)/(n-3)$ polyunsaturated fatty acid ratio from 2:50 to 1.68 brought about by a decrease in the proportion of 18 : 2 $(n-6)$ in concert with an increase in docosahexaenoic acid, 22 : 6 $(n-3)$. This decrease in the $(n-6)/(n-3)$ ratio was still apparent despite the increase in the proportion of arachidonic acid (20 : 4 $(n-6)$) for rats fed the sheep kidney fat diet. As a result of these changes, the unsaturation index (U.I.) increased from a value of 206 to 226 on feeding the sheep kidney fat diet.

Feeding the sunflower seed oil diet actually resulted in a small increase in the proportion of saturated fatty acids in the membrane phospholipids of rat heart membranes in comparison to that evident in the other two dietary groups. Although the proportion of polyunsaturated fatty acids was slightly increased over that evident for the other two dietary groups, the most significant changes were an increase in the $(n-6)$ and a decrease in the $(n-3)$ series of polyunsaturated fatty acids in comparison to the other two dietary groups. This resulted in an increase in the $(n-6)/(n-3)$ unsaturated fatty acid ratio, with the unsaturation index remaining essentially the same as that in the reference group. Changes in the individual fatty acids as a result of feeding the sunflower seed oil diet, included an increase in the proportion of 18 : 0 and 20 : 4 $(n-6)$ relative to the other two dietary groups, an increase in 18 : 2 $(n-6)$ (relatively to the sheep kidney fat group), and a decrease in the proportion of 16 : 0, 18 : 1 $(n-9)$, 22 : 6 $(n-3)$ and docosapentaenoic acid, 22 : 5 (n-3). The level of dimethyl acetal derivatives was not altered by the various dietary treatments.

The fatty acid composition of rat heart membrane phospholipids was also altered when the three experimental diets were supplemented with cholesterol (Table II). Feeding the reference plus cholesterol diet decreased the proportion of total saturated fatty acids and total polyunsaturated fatty acids, decreased the unsaturation index, and increased the value of the polyunsaturated to saturated and the $(n-6)$ to $(n-3)$ unsaturated fatty acid ratios in comparison to the reference diet alone. Changes in the proportion of individual fatty acids which gave rise to those changes in the reference plus cholesterol dietary group included a decrease in 16 : 0, 18 : 0, 20 : 4 $(n-6)$, 22 : 5 $(n-3)$ and 22 : 6 $(n-3)$, and a dramatic increase in 18 : 1 $(n-9)$.

Feeding the sheep kidney fat diet together with cholesterol increased the proportion of total saturated and polyunsaturated fatty acids, and the value of the unsaturation index, and decreased the value of the $(n-6)$ to $(n-3)$ polyunsaturated fatty acid ratio relative to the reference plus cholesterol diet. These changes were the result of

TABLE I

FATTY ACID COMPOSITION OF RAT HEART MEMBRANE PHOSPHOLIPIDS AFTER 21 WEEKS DIETARY FATTY ACID SUPPLEMENTATION

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 $(n-x)$ which refers to the first double bond counting from the terminal methyl group of the fatty acid. Fatty acid compositions of the P6000g–46000g membrane fraction are the mean relative percentage ± S.E. for $n = 4$ samples for each dietary group with the hearts of three animals for each sample. The unsaturation index (U.I.) is $\sum_{a=1}^{k}$ (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 compositional parameters for each dietary group were determined by Student's t-test. –,not determined and n.s., not significant.

Fatty acid	Diet			Significance, P		
	REF	SKF	SSO	REF vs. SKF	REF vs. SSO	SKF vs. SSO
14:0	tr.	tr.	tr.	–	–	–
15:0	tr.	tr.	tr.	–	–	–
DMA 16:0	1.4±0.1	0.8±0.1	1.3±0.2	–	–	–
16:0	13.2±0.1	10.5±0.2	9.6±0.2	< 0.001	< 0.001	< 0.02
17:0	0.5±0.1	0.5±0.1	tr.	–	–	–
DMA 18:0	0.8±0.1	1.5±0.2	1.1±0.1	–	–	–
18:0	21.0±0.3	24.3±0.2	26.1±0.1	< 0.001	< 0.001	< 0.001
18:1 ($n-9$)	9.6±0.1	9.8±0.4	6.3±0.1	n.s.	< 0.001	< 0.001
18:2 ($n-6$)	18.9±0.6	11.6±0.2	15.1±0.3	< 0.001	< 0.005	< 0.001
18:3 ($n-3$)	tr.	tr.	tr.	–	–	–
20:0	tr.	tr.	tr.	–	–	–
20:1	0.5±0.1	0.4±0.1	0.3±0.1	–	–	–
20:2 ($n-9$)	tr.	tr.	0.3±0.1	–	–	–
20:3 ($n-6$)	0.4±0.1	0.4±0.1	0.3±0.1	–	–	–
20:4 ($n-6$)	17.4±0.2	20.0±0.2	24.3±0.2	< 0.001	< 0.001	< 0.001
22:1/20:5 ($n-3$)	0.6±0.1	0.8±0.1	0.3±0.1	–	–	–
22:4 ($n-6$)	0.6±0.1	0.3±0.1	1.7±0.1	–	–	–
24:0	0.5±0.1	0.3±0.1	1.8±0.1	–	–	–
22:5 ($n-3$)	1.7±0.1	2.0±0.1	0.9±0.1	n.s.	< 0.001	< 0.001
22:6 ($n-3$)	12.5±0.1	17.0±0.1	10.2±0.1	< 0.001	< 0.001	< 0.001
Satd. (S)	37.5±0.4	37.9±0.4	40.1±0.2	n.s.	< 0.005	< 0.005
Unsatd.	62.5±0.4	62.1±0.4	59.9±0.2	n.s.	< 0.005	< 0.005
Poly (P)	52.4±0.4	51.9±0.4	53.3±0.2	n.s.	n.s.	< 0.025
($n-6$)	37.3±0.5	32.4±0.4	41.4±0.3	< 0.001	< 0.001	< 0.001
($n-3$)	14.9±0.2	19.3±0.1	11.6±0.1	< 0.001	< 0.001	< 0.001
DMA	2.2	2.3	2.3	–	–	–
P/S	1.40	1.37	1.33	–	–	–
($n-6$)/($n-3$)	2.50	1.68	3.57	–	–	–
U.I.	206	226	209	–	–	–

an increase in the proportion of 18:0, 20:4 ($n-6$) and 22:6 ($n-3$) and a decrease in 18:1 ($n-9$) and 18:2 ($n-6$) (Table II).

Compared to the sheep kidney fat dietary group without cholesterol, supplementation of this diet with cholesterol resulted in a decreased proportion of total saturated and polyunsaturated fatty acids, a decrease in the value of the unsaturation index and an increase in the value of the polyunsaturated to saturated and the ($n-6$) to ($n-3$) unsaturated fatty acids ratio. Changes in the proportion of the individual fatty acids which gave rise to these changes in the sheep kidney fat plus cholesterol dietary group included a decrease in 16:0, 18:0, 20:4 ($n-6$), 22:5 ($n-3$) and 22:6 ($n-3$) and a dramatic increase in 18:1 ($n-9$)

TABLE II

FATTY ACID COMPOSITION OF RAT HEART MEMBRANE PHOSPHOLIPIDS AFTER 13 WEEKS DIETARY CHOLESTEROL AND FATTY ACID SUPPLEMENTATION

Fatty acid compositions of the P6000 g–46000 g membrane fraction are the mean relative percentage ± S.E. for $n = 4$ samples from each dietary group with the hearts of three animals being used for each sample. Dietary groups are REF-C, reference diet (REF) plus cholesterol (CHOL); SKF-C, sheep kidney fat diet (SKF) plus cholesterol; SSO-C, sunflower seed oil diet (SSO) plus cholesterol. All other details are as described in Table I.

Fatty acid	Diet			Significance, P		
	REF +CHOL	SKF +CHOL	SSO +CHOL	REF-C vs. SKF-C	REF-C vs. SSO-C	SKF-C vs. SSO-C
14:0	tr.	tr.	tr.	–	–	–
15:0	tr.	tr.	tr.	–	–	–
DMA 16:0	1.7±0.1	1.2±0.1	1.1±0.1	–	–	–
16:0	8.5±0.3	7.9±0.2	6.7±0.2	n.s.	< 0.005	< 0.005
16:1 ($n-7$)	0.8±0.1	0.5±0.2	tr.	–	–	–
17:0	0.4±0.1	0.5±0.1	tr.	–	–	–
DMA 18:0	0.8±0.1	1.1±0.2	0.7±0.1	–	–	–
18:0	16.9±0.7	20.8±0.1	21.7±0.2	< 0.005	< 0.001	< 0.02
18:1 ($n-9$)	26.7±2.3	20.2±0.4	18.4±0.6	< 0.05	< 0.02	n.s.
18:2 ($n-6$)	18.6±0.8	13.8±0.1	19.0±0.5	< 0.001	n.s.	< 0.001
18:3 ($n-6$)	0.3±0.3	0.3±0.1	tr.	–	–	–
18:3 ($n-3$)	tr.	0.2±0.1	tr.	–	–	–
20:0	tr.	tr.	tr.	–	–	–
20:1	0.6±0.2	0.3±0.1	0.3±0.1	–	–	–
20:2 ($n-9$)	0.2±0.1	0.3±0.1	0.4±0.1	–	–	–
20:3 ($n-6$)	0.5±0.1	0.7±0.1	0.5±0.1	–	–	–
20:4 ($n-6$)	11.9±0.6	14.6±0.1	17.6±0.2	< 0.005	< 0.001	< 0.001
22:1/20:5 ($n-3$)	0.5±0.1	0.5±0.1	0.3±0.1	–	–	–
22:4 ($n-6$)	0.5±0.1	0.3±0.1	1.2±0.1	–	–	–
24:0	0.4±0.1	0.7±0.1	1.2±0.1	–	–	–
22:5 ($n-3$)	1.0±0.1	1.3±0.1	0.6±0.1	n.s.	< 0.001	< 0.001
22:6 ($n-3$)	9.3±0.4	14.3±0.1	9.4±0.1	< 0.001	n.s.	< 0.001
Satd. (S)	29.0±1.3	32.5±0.3	31.8±0.6	< 0.05	n.s.	n.s.
Unsatd.	71.0±1.3	67.5±0.3	68.2±0.6	< 0.05	n.s.	n.s.
Poly (P)	42.9±1.3	46.5±0.2	49.3±0.5	< 0.05	< 0.005	< 0.005
($n-6$)	32.0±0.9	30.0±0.1	38.9±0.5	n.s.	n.s.	< 0.001
($n-3$)	10.9±0.4	16.4±0.2	10.4±0.2	< 0.001	n.s.	< 0.001
DMA	2.52	2.29	1.86	–	–	–
P/S	1.48	1.43	1.55	–	–	–
($n-6$)/($n-3$)	2.93	1.83	3.73	–	–	–
U.I.	179	206	195	–	–	–

and less so for 18 : 2 ($n - 6$), in comparison to the same dietary group without added cholesterol. The changes occurring as a result of supplementing the sheep kidney fat diet with cholesterol were qualitatively the same as those induced by supplementation of the reference group with cholesterol (Table II).

Feeding the sunflower seed oil plus cholesterol diet increased the total proportion of the polyunsaturated and the ($n - 6$) series of unsaturated fatty acids as well as increasing the value of the polyunsaturated to saturated and the ($n - 6$) to ($n - 3$) unsaturated fatty acid ratio, and the unsaturation index compared to the reference diet plus cholesterol. Changes in the individual fatty acids which were apparent when feeding the sunflower seed oil plus cholesterol diet included a decrease in the proportion of 16 : 0, 18 : 1 ($n - 9$)

and 22:5 ($n-3$), and an increase in the proportion of 18:0 and 20:4 ($n-6$) relative to the reference plus cholesterol diet (Table II).

Compared to the sunflower seed oil dietary group without cholesterol, supplementation of this diet with cholesterol resulted in significant changes in the proportions of various individual fatty acids and changes in the values of the computational parameters which were qualitatively the same as those induced in the groups fed the reference diet plus cholesterol or the sheep kidney fat diet plus cholesterol compared to those animals on the diets without cholesterol.

The cholesterol and phospholipid content and the cholesterol to phospholipid ratio were also determined using the P6000g–46 000g cardiac membrane fraction. The effects of the various dietary lipid treatments with or without cholesterol supplementation are shown in Table III. For rats fed the reference diet or the reference diet plus cholesterol, no statistically significant differences were evident for the above parameters. However, a trend was apparent in which the cholesterol to phospholipid ratio in rats fed the cholesterol-supplemented diet was increased. This resulted from a decrease in the total phospholipid content of the membrane upon dietary cholesterol supplementation; total cholesterol content of the membrane remaining the same. When comparing the above parameters in rats fed the sheep kidney fat diet with or without cholesterol supplementation, there was a significant increase in the cholesterol to phospholipid ratio with the dietary cholesterol supplementation. This change was induced by a significant decrease in the total phospholipid content of the membrane despite the fact that total cholesterol also decreased as a result of the dietary cholesterol supplementation. For rats fed the sunflower seed oil diet with or without cholesterol, the result was similar to that with the sheep kidney fat diet, i.e. a significant increase in the cholesterol to phospholipid ratio was evident and was induced by a decrease in membrane phospholipid content. The total cholesterol content for cardiac membranes isolated from rats on each of the six dietary lipid treatments, was very similar (Table III).

TABLE III

EFFECT OF DIETARY LIPID AND CHOLESTEROL SUPPLEMENTATION ON LIPID CONTENT OF RAT HEART MEMBRANES

The lipid content of heart membranes was determined in the P6000g–46 000g membrane fraction. Data are presented as the mean ± S.E. for $n = 4$ samples per dietary group with each sample being obtained from the hearts of three animals. The significance, P, of differences between means was determined by Student's t-test. n.s., not significant

Diet	Cholesterol, C (nmol/mg protein)	Phospholipid, PL (nmol/mg protein)	Ratio C/PL (mol/mol)
REF	113 ± 3	482 ± 20	0.235 ± 0.008
REF + CHOL	117 ± 12	411 ± 26	0.283 ± 0.018
P	n.s.	n.s.	n.s.
SKF	131 ± 6	616 ± 32	0.212 ± 0.003
SKF + CHOL	105 ± 2	345 ± 9	0.307 ± 0.004
P	< 0.01	< 0.001	< 0.001
SSO	125 ± 9	560 ± 44	0.223 ± 0.002
SSO + CHOL	117 ± 6	343 ± 4	0.341 ± 0.009
P	n.s.	< 0.005	< 0.001

Total cholesterol levels (mg/100 ml) in the plasma in a similar group of rats fed the same abovementioned fatty acid-supplemented diets without cholesterol were (mean ± S.E., $n = 12$ animals per dietary group): REF, 99.0 ± 2.8; SKF, 106.5 ± 3.3; SSO, 94.7 ± 3.3 [38]. In this experiment, the total cholesterol levels in the plasma for the cholesterol supplemented groups, expressed as above for $n = 12$ animals per dietary group were: REF plus CHOL, 131.6 ± 4.2; SKF plus CHOL, 122.2 ± 1.9; SSO plus CHOL, 122.2 ± 4.8.

Adenylate cyclase studies

The effect of dietary lipid supplementation on adenylate cyclase activity and its stimulation by catecholamines and other activators was studied using a low-speed (P0–500g) cardiac membrane fraction. From other studies on rat (and marmoset monkey) heart adenylate cyclase [37], this particular cardiac membrane fraction was found to exhibit the highest-fold stimulation to the β-adrenergic agonist, isoproterenol, and to also exhibit the highest level of forskolin-stimulated adenylate cyclase activity. Because of these properties, this fraction was considered the most suited for comparing dietary lipid effects on catecholamine-

stimulated adenylate cyclase activity in rat heart tissue.

Dose-response curves for isoproterenol stimulation of adenylate cyclase activity in heart preparations from rats fed the various fatty acid-supplemented diets but without added cholesterol are shown in Fig. 1. Dose-response curves (plotted as specific activity), were very similar for all three dietary groups both in their response to isoproterenol (ED_{50} values remained in the range of $(1.36–3.98) \cdot 10^{-7}$ M), and in the activity of adenylate cyclase at maximum isoproterenol concentration. Some statistically significant differences between the sheep kidney fat group and the other two groups were apparent (see figure legend), but these were at concentrations of isoproterenol where the dose-response curves were at their steepest. In addition to these experiments at

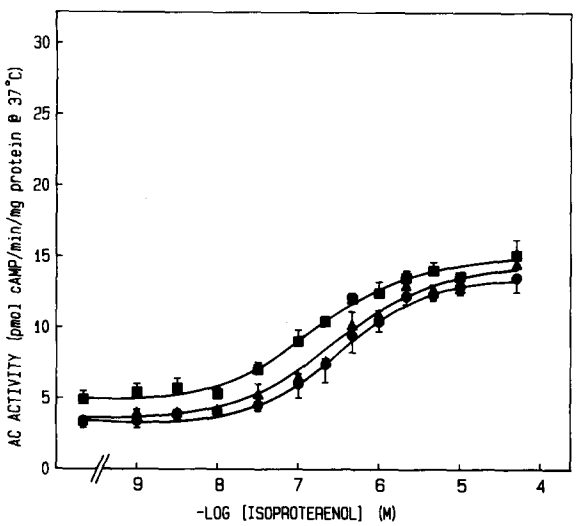

Fig. 1. Isoproterenol dose-response curve for cardiac adenylate cyclase (AC) activity for rats fed the REF (●); SKF (■) or SSO (▲) diets. Adenylate cyclase activity was determined on the low-speed (P0–500g) pellet as described in Methods. Data are presented as the mean ± S.E. for $n = 4$ animals per dietary group. Significant differences between the mean values at the various isoproterenol (Isop.) concentrations determined by Student's t-test were only evident when comparing REF versus SKF, $P < 0.05$, (10^{-9} M Isop.); $P < 0.02$, ($3.16 \cdot 10^{-8}$ M Isop.), and for SKF versus SSO, $P < 0.05$, (basal); $P < 0.05$, (10^{-8} M Isop.); $P < 0.025$, (10^{-7} M Isop.) and $P < 0.001$, ($2.15 \cdot 10^{-7}$ M Isop.). ED_{50} values for isoproterenol stimulation were calculated for each dietary group from normalized dose-response curves and were; $3.98 \cdot 10^{-7}$ M (REF); $1.36 \cdot 10^{-7}$ M (SKF); $3.41 \cdot 10^{-7}$ M (SSO).

21 weeks of dietary fatty acid supplementation, isoproterenol dose-response curves of the type shown in Fig. 1 have been repeated with rats maintained on the above same fatty acid-supplemented diets for 16 and 40 weeks, as well as with fresh and (−80 °C) frozen preparations of rat heart P0–500g membrane fractions isolated after each of the abovementioned periods of dietary fatty acid treatment. In all instances, the isoproterenol dose-response curves were virtually superimposable both in regard to the specific activity of the adenylate cyclase and the ED_{50} values for isoproterenol stimulation (data not shown).

In contrast to the results obtained with the dietary fatty acid supplements alone, isoproterenol dose-response curves for rats maintained on the fatty acid diets supplemented with cholesterol, exhibited greater differences in the specific activity of adenylate cyclase between the three dietary treatments (Fig. 2). For most concentrations of isoproterenol the sheep kidney fat plus cholesterol group was significantly higher than the sunflower seed oil plus cholesterol group, which in turn was higher than the reference plus cholesterol group (see legend Fig. 2).

The effect of the dietary lipid treatments on cardiac membrane adenylate cyclase activity was far more pronounced when a comparison was made of the effect of cholesterol supplementation on the individual fatty acid-supplemented diets and the reference diet. Isoproterenol dose-response curves for adenylate cyclase activity for rats fed the reference diet with or without cholesterol, are shown in Fig. 3. A significantly higher adenylate cyclase activity, particularly at higher concentrations of isoproterenol, was evident for the cholesterol supplemented group with no difference in the ED_{50} value for isoproterenol being apparent. The effects of cholesterol supplementation on isoproterenol-stimulated adenylate cyclase as well as other activators of adenylate cyclase, are also shown in Table IV for rats fed the reference diet. The significantly increased isoproterenol-stimulated adenylate cyclase activity in the cholesterol-supplemented group was also apparent for epinephrine and norepinephrine. ΔcAMP adenylate cyclase activity (see Table IV) was also increased in the cholesterol-supplemented group as was the forskolin-stimulated activity. Basal ac-

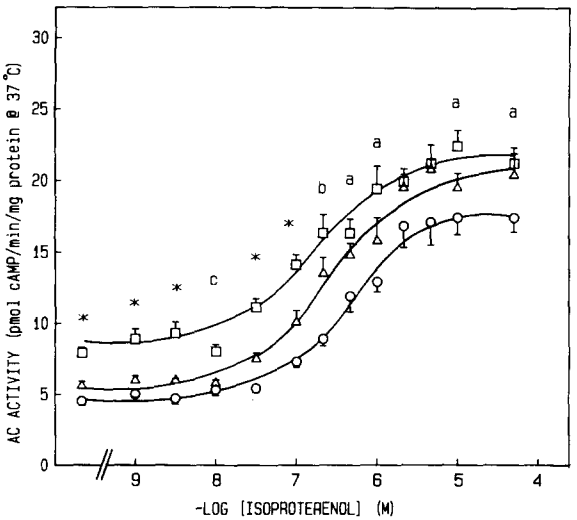

Fig. 2. Isoproterenol dose-response curve for cardiac adenylate cyclase (AC) activity for rats fed the REF + CHOL (○); SKF + CHOL (□) or SSO + CHOL (Δ) diets. Adenylate cyclase activity was assayed as described in Methods and is presented as in Fig. 1 for $n = 4$ animals per dietary group. Significant differences ($P < 0.05$ to $P < 0.001$), between the mean values at various isoproterenol concentrations (*) were evident when comparing REF versus SKF; REF versus SSO, and SKF versus SSO.Significant differences were also evident at *a*, REF versus SKF, $P < 0.05$ to $P < 0.01$; *b*, REF versus SSO, $P < 0.01$; *c*, SKF versus SSO, $P < 0.01$. ED_{50} values for isoproterenol stimulation were calculated for each dietary group from normalized dose-response curves and were; $3.98 \cdot 10^{-7}$ M (REF + CHOL); $1.59 \cdot 10^{-7}$ M (SKF + CHOL); $2.51 \cdot 10^{-7}$ M (SSO + CHOL).

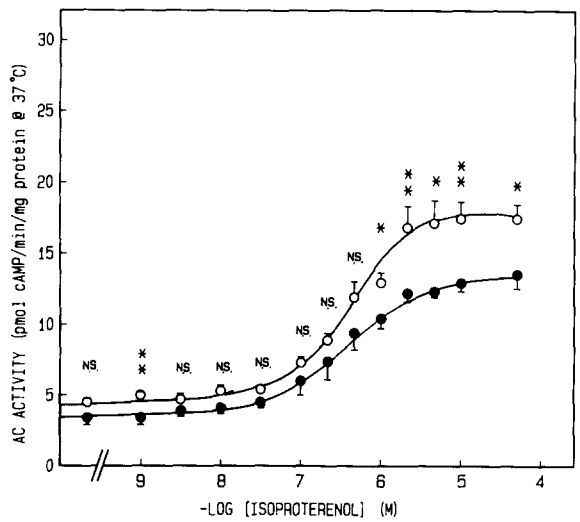

Fig. 3. Isoproterenol dose-response curve for cardiac adenylate cyclase (AC) activity for rats fed the REF (●) or the REF + CHOL diet (○). Adenylate cyclase activity was determined on the low-speed pellet P0–500*g*) pellet as described in Methods and data are shown as the mean ± S.E. for $n = 4$ animals per dietary group. The significance of differences between mean values at the various isoproterenol concentrations was determined by Student's *t*-test with * $P < 0.05$; ** $P < 0.025$; n.s., not significant. ED_{50} values for isoproterenol stimulation for each dietary group were calculated from normalized dose-response curves and were; REF, $3.98 \cdot 10^{-7}$ M; REF + CHOL, $3.98 \cdot 10^{-7}$ M.

TABLE IV

EFFECT OF DIETARY CHOLESTEROL SUPPLEMENTATION ON RAT 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 per dietary group. Activity was determined in the low-speed (P0–500*g*) membrane pellet as described in the Methods. The significance, *P*, of differences between means was determined by Student's *t*-test. n.s., not significant. ΔcAMP is the activity in the presence of $5 \cdot 10^{-5}$ M isoproterenol minus the basal activity.

Addition	Reference diet			Sheep kidney fat diet			Sunflower seed oil diet		
	−CHOL	+CHOL	*P*	−CHOL	+CHOL	*P*	−CHOL	+CHOL	*P*
Basal	3.4 ± 0.5	4.5 ± 0.3	n.s.	4.9 ± 0.6	7.9 ± 0.4	< 0.01	3.4 ± 0.1	5.6 ± 0.3	< 0.001
Propranolol (10^{-4} M)	3.8 ± 0.3	5.0 ± 0.2	< 0.02	5.0 ± 0.5	6.3 ± 0.3	n.s.	3.7 ± 0.3	5.5 ± 0.3	< 0.01
Isoproterenol ($5 \cdot 10^{-5}$ M)	13.5 ± 1.0	17.4 ± 1.0	< 0.05	15.1 ± 1.1	21.2 ± 1.1	< 0.01	14.5 ± 0.9	20.4 ± 1.5	< 0.02
ΔcAMP	10.5 ± 0.5	14.2 ± 1.2	< 0.05	10.9 ± 1.1	16.2 ± 0.9	< 0.01	11.6 ± 0.7	16.7 ± 0.8	< 0.005
Isoproterenol (10^{-5} M) propranolol (10^{-4} M)	3.9 ± 0.1	5.4 ± 0.4	< 0.02	5.5 ± 0.8	6.5 ± 0.4	n.s.	3.7 ± 0.2	5.7 ± 0.3	< 0.005
Epinephrine ($5 \cdot 10^{-5}$ M)	9.6 ± 0.5	13.2 ± 0.8	< 0.01	11.5 ± 0.7	18.6 ± 2.1	< 0.02	10.7 ± 0.8	17.1 ± 1.1	< 0.005
Norepinephrine ($5 \cdot 10^{-5}$ M)	10.4 ± 0.7	13.5 ± 1.0	< 0.05	11.3 ± 0.3	19.0 ± 2.0	< 0.01	11.2 ± 0.8	16.3 ± 0.9	< 0.01
NaF (10 mM)	17.3 ± 1.0	20.9 ± 1.3	n.s.	20.2 ± 1.0	28.7 ± 2.2	< 0.02	17.6 ± 0.4	26.9 ± 0.8	< 0.001
Forskolin (100 μM)	82.7 ± 2.5	91.9 ± 2.4	< 0.05	105 ± 7	99.0 ± 9	n.s.	78.4 ± 2.8	97.6 ± 6.0	< 0.05

tivity and isoproterenol-stimulated adenylate cyclase activity in the sheep kidney fat group was increased with cholesterol supplementation at all isoproterenol concentrations tested; once again the ED_{50} value for isoproterenol was not altered (Fig. 4). The ΔcAMP adenylate cyclase activity was increased in the cholesterol-supplemented group as were the epinephrine, norepinephrine and sodium fluoride-stimulated activities (Table IV). However, the forskolin-stimulated adenylate cyclase activity remained approximately the same in both dietary groups. As with the sheep kidney fat-supplemented group, cholesterol supplementation also resulted in an increase in basal and isoproterenol-stimulated adenylate cyclase activity in the sunflower seed oil group (Fig. 5). This significantly increased activity was evident at all isoproterenol concentrations. ED_{50} values for isoproterenol stimulation were not significantly different. ΔcAMP adenylate cyclase activity was increased in the cholesterol-supplemented group as was epinephrine, norepinephrine, sodium fluoride and forskolin-stimulated adenylate cyclase activities (Table IV). In the sunflower seed oil and

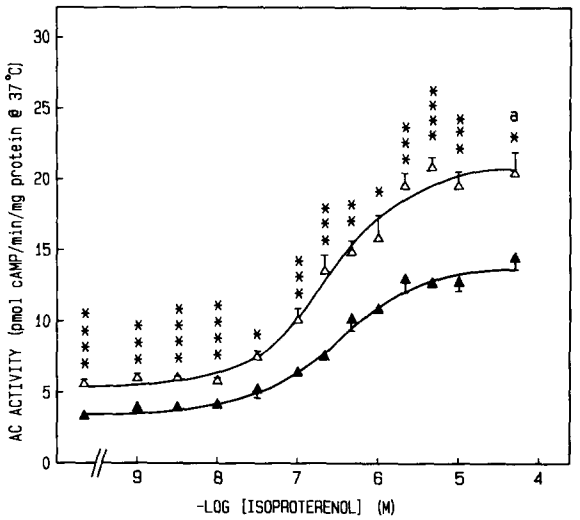

Fig. 5. Isoproterenol dose-response curve for cardiac adenylate cyclase (AC) activity for rats fed the SSO diet (▲) or the SSO + CHOL diet (△). Data are shown as the mean ± S.E. for $n = 4$ animals per dietary group. * $P < 0.05$; * a, $P < 0.02$; ** $P < 0.01$; *** $P < 0.005$; **** $P < 0.001$. ED_{50} values for isoproterenol stimulation were; SSO, $3.41 \cdot 10^{-7}$ M; SSO + CHOL, $2.51 \cdot 10^{-7}$ M. All other details are as described in Figs. 3 and 4.

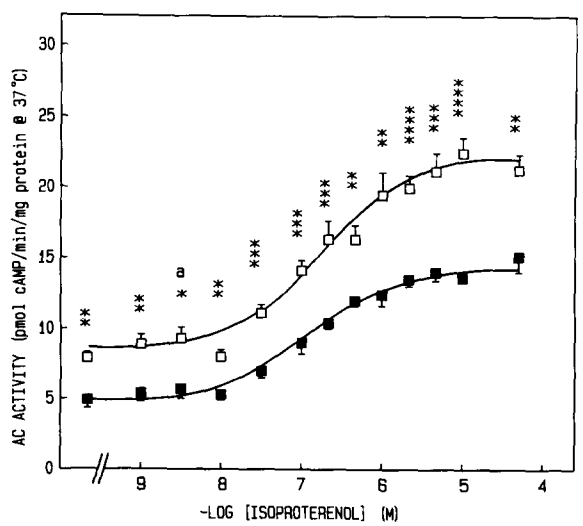

Fig. 4. Isoproterenol dose-response curve for cardiac adenylate cyclase (AC) activity for rats fed the SKF diet (■) or the SKF + CHOL, diet (□). Data are shown as the mean ± S.E. for $n = 4$ animals per dietary group. ** $P < 0.01$; * a, $P < 0.02$; *** $P < 0.005$; **** $P < 0.001$. ED_{50} values for isoproterenol stimulation were; SKF, $1.36 \cdot 10^{-7}$ M; SKF + CHOL, $1.59 \cdot 10^{-7}$ M. All other details are as described in Fig. 3.

the reference groups, adenylate cyclase activities in the presence of propranolol alone or with isoproterenol in the presence of excess propranolol, were also increased in the cholesterol-supplemented groups relative to those dietary groups not receiving the cholesterol supplement (Table IV).

β-Adrenergic receptor binding studies

β-Adrenergic receptor binding studies were performed on the high-speed cardiac membrane fraction (P6000g–46 000g) using the β-adrenergic radioligand [^{125}I](−)-iodocyanopindolol as described in the Methods. As mentioned previously, this particular membrane fraction exhibited the highest number of β-adrenergic receptors when compared with other membrane fractions prepared by differential centrifugation of cardiac homogenates [37]. Scatchard plots for the binding of [^{125}I](−)-iodocyanopindolol to rat heart membranes prepared from the various dietary groups, are shown in Fig. 6, and values for the percent specific binding, dissociation constant (K_d) and receptor number (B_{max}), are shown in Table V. Although

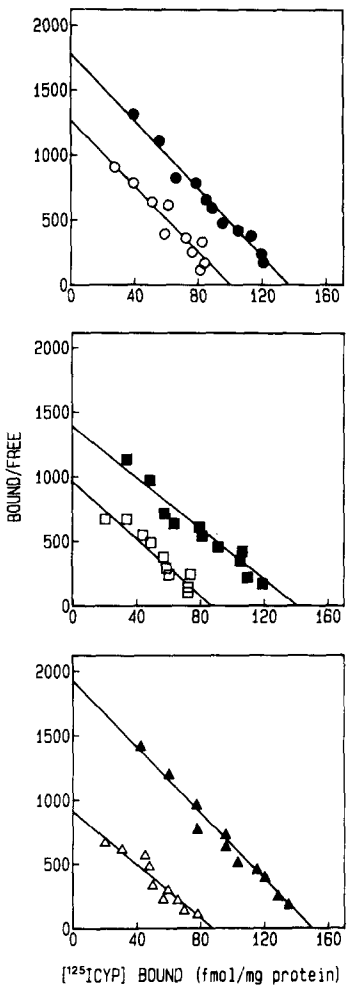

Fig. 6. Scatchard plots for [^{125}I](−)-iodocyanopindolol binding to rat heart membranes (P6000g–46000g fraction) after dietary fatty acid and cholesterol supplementation. Data are derived from at least three binding experiments per dietary group, with three hearts being pooled for the preparation of membranes for each experiment. Regression coefficients for each set of data points were ≥ -0.95. Dissociation constant, K_d (pM) and receptor number, B_{max} (fmol/mg protein), respectively, were: (●) REF, 76, 136; (○) REF+CHOL, 77, 98; (■) SKF, 97, 136; (□) SKF+CHOL, 90, 88; (▲) SSO, 76, 148; (△) SSO+CHOL, 92, 86.

values for the percent specific binding and the dissociation constant were not significantly different between all six dietary groups, the receptor number was reduced in those groups receiving the cholesterol supplement. This reduction in B_{max} value was statistically significant in the sheep kidney fat and sunflower seed oil groups receiving the cholesterol supplement relative to those same dietary groups not receiving cholesterol.

TABLE V

EFFECT OF DIETARY CHOLESTEROL AND FATTY ACID SUPPLEMENTATION ON β-ADRENERGIC RECEPTOR BINDING IN RAT HEART MEMBRANES

The dissociation constant (K_d) and the receptor number (B_{max}) were determined from Scatchard plots of [^{125}I](−)-iodocyanopindolol to the indicated number of samples (in brackets) of rat heart membranes from each dietary group. Binding experiments were done on samples of the high-speed (P6000g–46000g) membrane pellet with each sample being prepared from the hearts of three rats as described in Methods. The significance of differences between means ± S.E. was determined by Student's t-test. Differences in the dissociation constant were not significantly different when comparing any dietary group. Differences in receptor number were significant when comparing the SKF and SKF+CHOL dietary group ($P < 0.001$), and the SSO and SSO+CHOL dietary group ($P < 0.05$).

Dietary group		Specific binding (% at K_d)	K_d (pM)	B_{max} (fmol/mg protein)
REF	(4)	85±1	83± 7	140±18
REF+CHOL	(4)	83±3	102±18	110±11
SKF	(3)	83±3	123±33	151± 8
SKF+CHOL	(4)	85±1	92±13	85± 3
SSO	(3)	86±1	85±16	153±29
SSO+CHOL	(4)	84±2	82±18	82± 7

Relationship between cholesterol/phospholipid ratio, adenylate cyclase activity and β-adrenergic receptor number in cardiac membranes

An increase in the cholesterol/phospholipid ratio of cardiac membrane lipids as a result of the various dietary lipid treatments was positively correlated with an increase in ΔcAMP adenylate cyclase activity, as well as isoproterenol, epinephrine and norepinephrine-stimulated adenylate cyclase activity and negatively correlated with β-adrenergic receptor number (Fig. 7). The above parameters exhibited high correlation coefficients over the range of values for membrane cholesterol/phospholipid ratio of 0.21 to 0.34.

Discussion

The results of this study clearly show that in the rat, dietary cholesterol with or without fatty

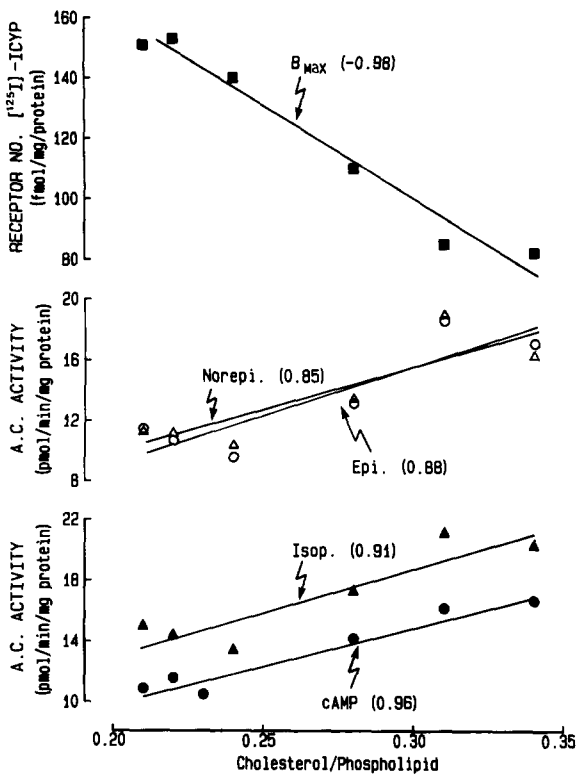

Fig. 7. The effect of dietary alteration of rat heart membrane cholesterol to phospholipid ratio on β-adrenergic receptor number and catecholamine-stimulated adenylate cyclase (AC) activity. β-Adrenergic receptor number ((−)-iodocyanopindolol binding) (■); activity of adenylate cyclase in the presence of $5 \cdot 10^{-5}$ M norepinephrine (△); $5 \cdot 10^{-5}$ M epinephrine (○); $5 \cdot 10^{-5}$ M isoproterenol (▲); ΔcAMP adenylate cyclase activity (defined as in Table IV) (●). Numbers in brackets are the correlation coefficients for each data set.

acid supplementation had a far greater effect on the membrane lipid composition and subsequently on the activity of the membrane-associated β-adrenergic adenylate cyclase system of the heart, than did dietary fatty acid supplementation alone. Although the fatty acid-supplemented diets produced significant changes in the cardiac membrane polyunsaturated fatty acid profile particularly with regard to the $(n-6)$ to $(n-3)$ ratio, they had little effect on either the value of the membrane cholesterol to phospholipid ratio, the activity of isoproterenol-stimulated adenylate cyclase, or the affinity and number of β-adrenergic receptor sites. This is in agreement with our previous experiments with rats fed dietary fatty acid supplements of the type used in this study [7]. In contrast to those rats fed only the fatty acid supplements, dietary cholesterol supplementation resulted in significant effects on the value of the membrane cholesterol to phospholipid ratio, the activity of the catecholamine-stimulated adenylate cyclase system, and the number of β-adrenergic receptor sites.

These dietary-induced changes in adenylate cyclase activity are consistent with the results from other studies which show that this enzyme-receptor complex is sensitive to certain changes in membrane lipid compositional characteristics, particularly those which influence membrane physico-chemical properties [8,9,27,29]. This sensitivity to membrane properties may derive from the fact that some or all of the components of hormone-sensitive adenylate cyclases are believed to undergo some lateral diffusion in the membrane during the process of transmembrane signalling [39,40]. Confirmation that hormone-sensitive adenylate cyclases are influenced by membrane physico-chemical properties has come from studies using in vivo modification of membrane lipid composition by dietary lipid supplementation [10,11,28,31,41], modification of the membrane lipid composition of cultured cell lines [42,43], in vitro lipid modification of isolated cell membrane preparations containing hormone-receptor adenylate cyclase components [30,44], reconstitution experiments [45], and by introducing lipid-perturbating agents into membranes to induce changes in membrane physical properties [46,47]. These studies have shown that a number of lipid compositional changes, such as changes in the proportions of the various phospholipid classes in the membrane [42,43] and changes in acyl fatty acid composition [10,42,43], can influence the activity of hormone-sensitive adenylate cyclases. Furthermore, these adenylate cyclases have been shown to be particularly sensitive to changes in membrane cholesterol, principally alterations in the value of the membrane cholesterol to phospholipid ratio [8,9,11,30].

In the present study, catecholamine-stimulated adenylate cyclase activity associated with rat heart was directly proportional to the value of the membrane cholesterol to phospholipid ratio. Although

we did not measure the relative fluidity of cardiac membrane lipids isolated from rats on the differing dietary lipid treatments, the value of the cholesterol to phospholipid ratio has been related to the degree of lipid ordering in the membrane determined by appropriate biophysical (e.g. fluorescent probe) techniques; this latter factor is known to influence the activity of hormone-sensitive adenylate cyclase [8,9,30]. This would therefore imply that in this study, the increased activity of the catecholamine-stimulated adenylate cyclase following dietary cholesterol supplementation, was a reflection of increased lipid ordering consequent upon an increase in the cholesterol to phospholipid ratio in the membrane. However, it has also been observed that changes in the cholesterol to phospholipid ratio of rat liver plasma membranes leads to both an increase and a decrease in the activity of the glucagon-stimulated adenylate cyclase, there being an optimum cholesterol to phospholipid ratio for the expression of maximum enzyme activity [9]. These results and those of our own therefore confirm that adenylate cyclase and its components such as hormone receptor and guanine nucleotide regulatory proteins, are modulated by the nature of their surrounding membrane lipid environment, with changes in the molecular ordering of the associated membrane lipids probably being of critical importance. Furthermore, they extend the above findings to the catecholamine-stimulated adenylate cyclase system of the heart both for the rat and the marmoset monkey [7].

Although the dietary cholesterol treatment increased catecholamine-stimulated adenylate cyclase activity in the rat heart, it did not appreciably affect the affinity of the β-adrenergic receptor as measured by (−)-iodocyanopindolol binding. However, there was a dramatic decrease in β-adrenergic receptor number accompanying the increase in adenylate cyclase activity. This decrease in receptor number may reflect down regulation of the β-adrenergic receptor [24] in response to the increased responsiveness of adenylate cyclase to catecholamine stimulation following dietary cholesterol supplementation. In other studies where we have observed an increase in cardiac catecholamine-stimulated adenylate cyclase activity, such as marmoset monkeys fed a high saturated fatty acid-supplemented diet, we have also observed a concomitant decrease in β-adrenergic receptor number [7]. This may represent an attempt by the organism to maintain homeostasis at the receptor level, in the face of an increased responsiveness of the adenylate cyclase system to stimulation by catecholamines. The failure to significantly after β-adrenergic receptor affinity by dietary lipid treatment agrees with findings from other studies [31], in which isoproterenol-stimulated cAMP accumulation in erythrocytes from cholesterol-fed quails was significantly elevated, despite the fact that β-adrenergic receptor affinity was not altered. Loh and Law [27] 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 following dietary cholesterol supplementation, were primarily mediated by the effect of membrane lipids on the functioning of certain post-receptor mechanisms.

Although there were significant differences in cardiac membrane phospholipid fatty acid composition, both as a result of the dietary fatty acid supplements and the dietary cholesterol treatment, it would appear that these changes per se were not sufficient to significantly influence the activity of the membrane-associated catecholamine-stimulated adenylate cyclase. This is supported by the fact that the dietary fatty acid treatments in the cholesterol and non-cholesterol dietary groups, did not significantly influence the proportion of saturated or unsaturated fatty acids in the cardiac membrane phospholipids. Furthermore, in these groups, the dietary fatty acid 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 relative to the low mixed-fat reference diet. These dietary fatty acid treatments have also been shown not to significantly alter cardiac membrane phospholipid headgroup composition in the rat [20,21]. The failure to significantly alter membrane lipid saturation/unsaturation or phospholipid headgroup composition, together with the similarity of

changes in the $(n-6)/(n-3)$ ratio of polyunsaturated fatty acids in the cardiac membranes from both cholesterol- and non-cholesterol-supplemented groups as a result of the various dietary fatty acid treatments, would suggest that the only unique membrane lipid modification was the altered cholesterol to phospholipid ratio. As mentioned previously, alterations in this parameter may have altered cardiac membrane lipid physical properties and influenced catecholamine-stimulated adenylate cyclase activity.

As has already been reported with rats [14,17] and marmoset monkeys [7,18], there is considerable homeostasis in the membrane lipid composition despite large differences in the nature of the dietary fatty acid intake. This is particularly evident in the maintenance of a relatively constant proportion of membrane lipid saturation and unsaturation. Dietary-induced changes in the membrane phospholipid fatty acid composition in the particular cardiac membrane preparation used in this study which exhibited the highest β-adrenergic receptor binding activity [37], was qualitatively similar to those previously reported for purified heart mitochondrial membranes from rats fed these same fatty acid-supplemented diets [5,6,12,17]. 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 supplements [5,12, 17].

Although the maintenance of a relatively constant level of membrane lipid saturation and unsaturation was also observed with rats fed the combined cholesterol and fatty acid supplements, there were some significant differences in membrane lipid composition between the cholesterol- and non-cholesterol-supplemented groups besides the altered membrane cholesterol to phospholipid ratio. Irrespective of the particular fatty acid supplement, the proportion of total saturated and polyunsaturated fatty acids in the cholesterol-supplemented group was decreased while the proportion of oleic acid was increased in comparison to the respective dietary groups not receiving the cholesterol supplement. This observation also suggests that a further homeostatic mechanism of the type described by Sinensky [48], is operative. This homeostatic mechanism may attempt to maintain an equivalent lipid viscosity or lipid ordering in the membrane in the face of potential physicochemical changes in the membrane resulting from the altered membrane cholesterol to phospholipid ratio. Given that an increase in the value of the cholesterol to phospholipid ratio leads to an increase in membrane lipid ordering, this could be partially offset by a decrease in the proportion of membrane saturated fatty acids to a level still compatible with function, together with an increase in the number of acyl fatty acids containing at least one *cis* double bond. The contribution of double bonds to membrane lipid disorder or fluidity is not proportional to the number of double bonds in the fatty acyl chain, but rather becomes less and less significant after the inclusion of the first and subsequent *cis*, methylene-interrupted double bonds [2]. Hence a dramatic increase is apparent in the proportion of oleic acid in the membrane lipids. The decrease in membrane polyunsaturated fatty acids is probably a reflection of the requirement to incorporate into the membrane phospholipids more acyl fatty acids containing one *cis* double bond such as oleic acid, to maximize the extent of membrane lipid disorder.

The increase in the cardiac membrane cholesterol to phospholipid ratio evident with dietary cholesterol was brought about by a reduction in the phospholipid content (on a mg protein basis), rather than by any significant change in the amount of cholesterol in the membrane. This would imply that a change in the total lipid to protein ratio in the membrane may have occurred as a result of the cholesterol supplementation. While this result is unexpected, it is not unprecedented as indicated from the data of Morson and Clandinin [10] for rat liver plasma membranes, Thomson et al. [49], for rat intestinal brush-border membranes, and Brasitus et al. [50], for rat intestinal microvillus membranes. In the first of these studies, feeding a high linoleic acid diet reduced the absolute amounts (on a mg membrane protein basis) of cholesterol, phosphatidylcholine and phosphatidylethanolamine compared to a low linoleic acid diet. Furthermore, Morson and Clandinin [10] reported that the plasma mem-

brane cholesterol content was directly related to the sum of the phosphatidylcholine plus phosphatidylethanolamine in the membrane. As the amount of the other major component in the membrane, sphingomyelin, was not altered by the dietary linoleic acid treatment, these results can be interpreted as an indication of a dietary-induced change in the lipid to protein ratio of rat liver plasma membranes. In the study by Thomson et al. [49], brush-border membranes isolated from the jejunum of rats fed a high saturated fatty acid diet in comparison to a high polyunsaturated fatty acid diet, exhibited a decreased membrane cholesterol to phospholipid ratio which was brought about solely by a decrease in the membrane total phospholipid content (on a mg membrane protein basis). This would suggest that, as observed in our own study, changes in the membrane cholesterol to phospholipid ratio, can be induced by changes in both membrane cholesterol and phospholipid content and that changes in the membrane lipid to protein ratio can occur. Finally in the developmental study by Brasitus et al. [50], they report that in rat microvillus membranes isolated from the proximal half of the intestine, there was a significant decrease in the membrane lipid to protein ratio at 117 weeks of age when compared to 6 weeks of age. Taken together, the above studies suggest that developmental and nutritional factors are likely to affect the absolute amount as well as the types of lipid present in certain cellular membranes. Such a change could further alter membrane lipid biophysical properties in much the same manner as a change in the membrane cholesterol to phospholipid ratio.

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