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EVIDENCE FOR A DECREASE IN THE EFFICIENCY OF β-RECEPTOR COUPLING TO ADENYLATE CYCLASE IN LIVER MEMBRANES FROM SUCROSE-FED RATS

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Abstract—Sucrose feeding has been shown previously to alter the plasma concentration of several factors which may regulate β-adrenergic receptors, including corticosteroids and insulin as well as altered sympathetic nervous system (SNS) tone. For this reason we initiated a study of the effects of sucrose feeding on the β-adrenergic receptor-adenylate cyclase system in rat liver plasma membranes. β-Adrenergic responsiveness was monitored by measuring isoproterenol stimulation of adenylate cyclase activity, while β-adrenergic receptor characteristics were evaluated by analyzing [^{125}I]iodocyanopindolol ([^{125}I]CYP) binding. Rats fed rat chow *ad lib.* supplemented by drinking water containing 10% sucrose solution exhibited a 50–75% reduction in hepatic isoproterenol-sensitive adenylate cyclase activity. This effect of sucrose was also observed in adrenalectomized (ADX) and 6-hydroxydopamine-pretreated animals, ruling out a causal role for corticosteroids or the sympathetic nervous system respectively. No effect was observed on basal, glucagon-, fluoride- or GTP-stimulated adenylate cyclase. A small but significant decrease in [^{125}I]CYP specific binding capacity was observed in liver membranes prepared from sucrose-fed ADX rats, whereas no change in [^{125}I]CYP binding capacity was observed in in sucrose-fed normal rats. These observations suggest that β-receptor to adenylate cyclase coupling efficiency is decreased by the sucrose diet. The activities of two membrane-associated phospholipid methyltransferases and the content of endogenous *S*-adenosylmethionine in liver were reduced by sucrose feeding, implying a defect in the methylation pathway for phosphatidylcholine synthesis. The possible relationship between this latter finding and the observed decrease in β-adrenergic receptor to adenylate cyclase coupling efficiency is discussed.

In rat liver, unlike the liver of other species including man, adrenergic stimulation of glycogenolysis and glucose release is mediated predominantly via α_1-adrenergic receptors operating by a cyclic AMP-independent mechanism (see Ref. 1 for review). Nevertheless, there is a significant beta component which contributes to the sympathetic stimulation of hepatic glucose output in rat liver. The relative importance of β-receptors in mediating adrenergic responsiveness in this system is not fixed, but rather is dependent on or regulated by various factors including age, sex and diet, as well as adrenal and thyroid status [2–6]. It has been shown that sucrose added to animal diets can increase the plasma concentration and activity of several potential regulators of β-adrenergic receptors and/or β-receptor mediated responses. These regulators (see Refs. 7–9 for review) include corticosteroids, fasting insulin levels and the sympathetic nervous system [10, 11]. We hypothesized that sucrose-feeding might induce demonstrable alterations in hepatic β-adrenergic events. In this paper, we describe the effects of a sucrose-supplemented diet on the hepatic β-adrenergic receptor-adenylate cyclase system. The diet was administered as previously described [11] by substituting drinking water with a 10% sucrose solution to which animals were allowed continual free access in addition to *ad lib.* rat chow.

In theory, regulation of β-adrenergic responsiveness can occur: (a) at the receptor level via changes in β-receptor number of affinity; (b) at the effector level (i.e. adenylate cyclase) via increases or decreases in adenylate cyclase specific activity; (c) secondary to a defect in β-receptor coupling to effector; or (d) a combination of these [7, 8]. We employed the β-receptor radioligand [^{125}I]CYP§ to monitor β-adrenergic receptors on partially purified liver cell plasma membranes. Isoproterenol stimulation of membrane bound adenylate cyclase was measured as an index of β-adrenergic responsiveness.

We report that 2 weeks of feeding a sucrose diet resulted in a decrease in hepatic β-receptor to adenyl-

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§ Abbreviations: [^{125}I]CYP, [^{125}I]iodocyanopindolol; SNS, sympathetic nervous system; 6-OHDA, 6-hydroxydopamine; PMT I, phospholipid methyltransferase I; PMT II, phospholipid methyltransferase II; AdoMet, *S*-adenosyl-L-methionine; AdoHcy, *S*-adenosylhomocysteine; ADX, adrenalectomized; and HPLC, high performance liquid chromatography.

ate cyclase coupling efficiency which may be secondary to diet-induced changes in plasma membrane phospholipid synthesis or composition.

MATERIALS AND METHODS

Materials. Cyanopindolol was a gift from Dr. D. Hoyer (Sandoz Pharmaceuticals, Switzerland). *S*-Adenosyl-L[*methyl*-^{3}H]methionine was purchased from ICN (Irvine, CA). [α-^{32}P]Adenosine 5′-triphosphate, [^{3}H]cyclic AMP and Na^{125}I were obtained from New England Nuclear (Boston, MA). Dowex AG-50 W-X4 (200–400 mesh) cation-exchange resin was purchased from Bio-Rad Laboratories (Richmond, CA). All other chemicals were purchased from the Sigma Chemical Co. (St. Louis, MO).

Animal treatments. C-D strain female albino rats (175–275 g), purchased from Charles River Laboratories, were maintained *ad lib.* on Purina Formulab Rat Chow No. 5008 and either plain water (control diet) or 10% (w/w) sucrose (sucrose diet) for 2 weeks as indicated. Bilaterally adrenalectomized animals were maintained on rat chow and normal saline (control diet) or 10% (w/w) sucrose in normal saline (sucrose diet) for 2 weeks as indicated. Animals maintained on the sucrose diet ate 25% less rat chow, but this decrease was compensated for by an isocaloric increase in sucrose-solution consumption over the 2-week period of observation. As a result, no difference was observed in the body weights of the control and sucrose-fed groups.

In some experiments, animals were injected with 6-OHDA which produced a chemical sympathectomy. 6-OHDA was dissolved immediately before use in a vehicle of ice-cold normal saline (pH adjusted to 3.0 with HCl) and ascorbic acid (1 mg/ml) constantly bubbled with 100% N_2. Unfasted rats were injected via the tail vein with an initial dose of 60 mg/kg (1 ml/kg) 6-OHDA followed 24 hr later by a second dose injection of 100 mg/kg (1 ml/kg) 6-OHDA. Two hours after the second 6-OHDA injection, half of the animals were placed on the sucrose-supplemented diet while the other half remained on the control diet. Two weeks later, a portion of the right liver lobe of the animals in each group was removed and frozen in liquid N_2 while the remainder of the liver was used for plasma membrane preparation. The portion frozen in liquid N_2 was stored at −70° until being assayed for endogenous norepinephrine (NE) content by a modification of the method of Eriksson and Persson [12]. Two weeks following 6-OHDA treatment, an 87% reduction in endogenous hepatic NE content of control- and sucrose-fed animals was observed (data not shown), verifying that the chemical sympathectomy was relatively complete.

Adenylate cyclase assay. Adenylate cyclase activity was measured as described by Salomon [13], using partially purified liver cell plasma membranes. The plasma membranes were prepared according to the method of Neville [14] up to step 11 as modified by Pohl *et al.* [15]. Membranes were prepared simultaneously from control or sucrose-fed animals, diluted in 1 mM $NaHCO_3$ buffer, and stored in liquid nitrogen for no more than 2 weeks prior to assay. The adenylate cyclase assay medium contained 0.5 mM [^{32}P]ATP (0.5–4 × 10^6 cpm), 5 mM magnesium acetate, 0.05 mM cyclic AMP, 1 mM dithiothreitol, 0.1 mg/ml bovine serum albumin, 5 mM creatine phosphate, 50 units/ml creatine phosphokinase, 25 mM Tris acetate (pH 7.6 at 30°), 0.01 mM GTP (unless otherwise indicated), and 40–50 μg of membrane proteins in a final volume of 100 μl. Incubation at 30° in a shaking water bath for 15 min was initiated by the addition of membranes and terminated by the 200-μl addition of "stopping solution" [2% sodium lauryl sulfate, 45 mM ATP, 1.3 mM cyclic AMP, (pH 7.5)] followed by 4 min of boiling. Radiolabeled cyclic AMP was isolated and yield was calculated as described in detail by Salomon [13]. Samples were counted in Redisolv-EP (Beckman Corp, Palo Alto, CA), using a Packard liquid scintillation counter. Protein was determined by the procedure of Lowry *et al.* [16] using a standard of bovine serum albumin. Fluoride- or glucagon-sensitive adenylate cyclase activity was measured in every experiment as a positive control.

[^{125}I]CYP binding assay. The β-antagonist CYP was radioiodinated and purified as described by Engel *et al.* [17] using carrier-free Na^{125}I. [^{125}I]CYP was purified by descending paper chromatography and recovered as a single peak 5–10 cm from the origin. Binding of [^{125}I]CYP to liver cell plasma membrane β-receptors was studied using the method of El-Refai and Chan [4] with some minor modifications. The assay medium contained 50 mM Tris buffer (pH 7.6 at 37°), 150 mM NaCl and 12–20 μg of plasma membrane protein in an incubation volume of 300 μl. Following a 60-min incubation at 37° in a shaking water bath, samples were diluted to 10 ml with a room temperature buffer containing 10 mM Tris (pH 7.6) in 150 mM NaCl and rapidly filtered, under reduced pressure, through 24 mm Whatman GF/C glass fiber filters. The filters were washed with an additional 10 ml of the dilution buffer, transferred to test tubes and counted in a Packard gamma counter. The filtration and wash steps were completed in less than 30 sec. Non-specific binding was determined by including 2 μM (*d*, *l*)-propranolol in the assay mixture, and specific binding (80–90% of the total binding) was defined as the difference between total and non-specific binding.

Determination of phospholipid methyltransferase I and II activities and endogenous S-adenosylmethionine and S-adenosylhomocysteine levels. Activities of the two plasma membrane associated phospholipid methyltransferases (i.e. PMT I and PMT II) were measured according to the method of Sastry *et al.* [18] with minor modifications [19, 20]. The 800 μl assay medium contained 50 mM Tris-HCl buffer (pH 8.0 at 37°), 10 mM $MgCl_2$, 0.1 mM EDTA and 200–300 μg of plasma membrane proteins. Two concentrations of AdoMet were employed to differentiate between the two membrane associated phospholipid methyltransferase (i.e. 0.5 μM AdoMet for PMT I and 200 μM AdoMet for PMT II) and [^{3}H-*methyl*]AdoMet was employed as a radiotracer (1 μCi/tube). Incubations at 37° for 30 min were initiated by the addition of AdoMet and terminated by the addition of 4 ml of a 2/1/0.02 (by vol) mixture of chloroform/methanol/HCl. This mixture was shaken vigorously for 10 min and washed twice with 2 ml of

Table 1. Lack of effect of sucrose diet on basal, glucagon-stimulated and azide-enhanced/flouride-stimulated adenylate cyclase activity*

	Adenylate cyclase activity [pmoles cAMP·(mg protein)$^{-1}$·min^{-1}]			
	Basal†	Basal (+ GTP)	Glucagon (0.28 μM)	NaF (10 mM)+ NaN_3 (20 mM)†
Control	7.8 ± 0.3	38.3 ± 3.6	150.5 ± 19.0	127.4 ± 14.5
Sucrose-fed	9.6 ± 1.3	39.9 ± 4.2	169.4 ± 35.3	138.5 ± 16.5
ADX	ND‡	55.0 ± 10.2	145.0 ± 25.4	129.4 ± 6.2
Sucrose-fed/ADX	ND	53.4 ± 4.4	147.3 ± 5.2	119.5 ± 5.5

* Values represent means ± S.E. of values from three to four separate experiments using different membrane preparations.
† Assayed in the absence of GTP.
‡ Not determined.

0.1 M KCl in 50% methanol. The upper fluid layer was discarded after each wash. An aliquot of the chloroform layer was separated by HPLC (as described by Chen and Kou [21]) into phosphatidylcholine and its dimethyl- and monomethyl precursors. Samples of the total phospholipid fraction and each separated peak were transferred to a scintillation vial, evaporated to dryness, and dissolved in 10 ml of Aquasol (Amersham Corp., Arlington Heights, IL), and total radioactivity was measured. The phospholipid methyltransferase activities are expressed as picomoles of ^{3}H-methyl transferred per mg of protein per 30 min. Hepatic Adomet and AdoHcy were determined using the isotope-dilution/ion exchange/HPLC method described by Schatz *et al.* [22].

Significance of data. Text statements about changes refer only to those shown to be significant by Student's *t*-test with minimal significance defined as $P < 0.05$. Figures show the data from experiments repeated two or more times with similar results.

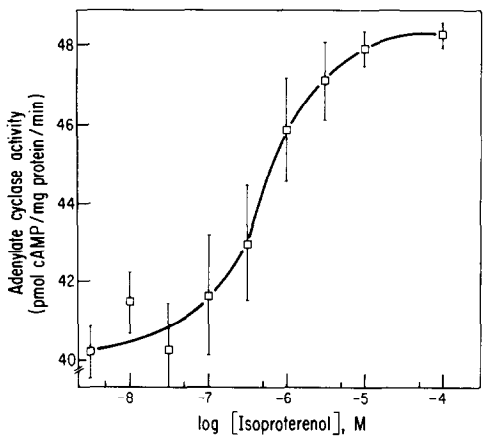

Fig. 1. Concentration dependence of isoproterenol stimulation of adenylate cyclase activity. Partially purified liver cell plasma membranes were assayed for adenylate cyclase activity as described in the text in the presence or absence of various concentrations of (*l*)-isoproterenol. Incubations were for 15 min at 30°.

RESULTS

Effect of sucrose feeding on isoproterenol stimulation of adenylate cyclase. In initial experiments (see Table 1), the influence of GTP, glucagon and a fluoride–azide mixture on adenylate cyclase activity was measured and found to be similar to previously reported values [7, 8, 23]. In comparison to the agents above, isoproterenol stimulation of adenylate cyclase in adult rat liver membranes was small, but activity increased in a dose-dependent manner (Fig. 1), and this increase was not observed in the presence of the beta-receptor antagonist propranolol (Fig. 2).

Membranes from rats that had been maintained on sucrose feeding for 2 weeks showed a 50–75% reduction in isoproterenol-sensitive adenylate cyclase activity (Fig. 2) as compared to control rats

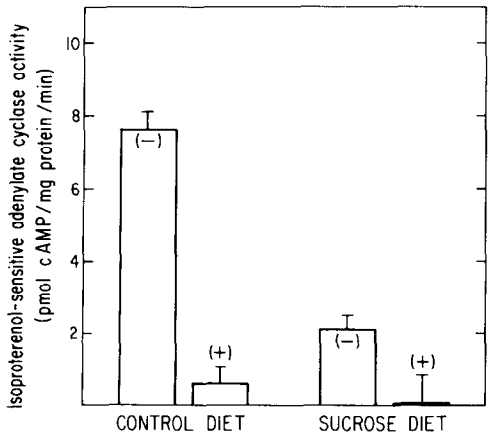

Fig. 2. Effect of sucrose feeding on isoproterenol-sensitive adenylate cyclase activity. Isoproterenol-sensitive cyclase activity is defined as the increase in activity, above basal, stimulated by isoproterenol (5×10^{-5} M). The bars show the mean ± S.E. values from a representative experiment performed in the presence (+) or absence (−) of 5×10^{-7} M (*d,l*)-propranolol. Results shown are from an assay performed simultaneously on membranes from control and sucrose-fed animals with respective basal activities of 27.0 ± 2.0 and 30.1 ± 0.7 pmoles cAMP formed·(mg protein)$^{-1}$·min^{-1}.

Table 2. Effect of 6-OHDA and sucrose feeding on isoproterenol-sensitive adenylate cyclase activity

Injection	Diet	Isoproterenol-sensitive adenylate cyclase activity* [pmoles cAMP·(mg protein)$^{-1}$·min^{-1}]
Sham	Control	7.06 ± 0.40
Sham	Sucrose	3.93 ± 0.10†
6-OHDA	Control	6.66 ± 0.80
6-OHDA	Sucrose	3.66 ± 1.0‡

* Isoproterenol-sensitive adenylate cyclase activity was assayed under conditions identical to those in the legend of Fig. 2.
† Significantly lower than sham-injected control diet ($P < 0.05$).
‡ Significantly lower than 6-OHDA-injected control diet ($P < 0.05$).

maintained on normal chow. Sucrose feeding had no effect on basal, glucagon- or fluoride/azide-stimulated adenylate cyclase activity (Table 1).

To test the hypothesis that the decreased β-adrenergic responsiveness observed above was secondary to a sustained increase in sympathetic nervous system tone induced by sucrose feeding [11], animals were chemically sympathectomized with 6-OHDA and then fed the control or sucrose diet for 2 weeks. No significant differences were observed in basal or isoproterenol-sensitive adenylate cyclase activities in hepatic plasma membranes from 6-OHDA—or sham-injected rats fed the control diet. Furthermore, chemical sympathectomy did not protect against the inhibitory effect of sucrose feeding on beta-adrenergic responsiveness (Table 2).

Bilateral adrenalectomy has been reported previously to increase isoproterenol-sensitive cyclase activity in rat hepatic membranes [5], and the results shown in Fig. 3, as compared to Figs. 1 and 2, confirm this finding. The inhibitory effect of sucrose feeding on isoproterenol stimulation of adenylate cyclase activity was also observed in membranes from ADX animals but to a lesser fractional extent than that observed in normal animals. Thus, when measured using maximally effective isoproterenol concentrations (i.e. above 10 μM), isoproterenol-sensitive adenylate cyclase activity in membranes from sucrose-fed ADX rats was reduced by approximately 20–40% as compared to the 50–75% reduction observed in sucrose-fed normal animals when compared to the appropriate non-sucrose fed group. A 2 to 3-fold shift to the right in the isoproterenol dose–response curve was seen in adenylate cyclase experiments comparing liver membranes from control to sucrose-fed rats (Fig. 3).

Effect of sucrose feeding on this specific binding of [^{125}I]*CYP to β-receptors.* To test the hypothesis that the effects of sucrose feeding on isoproterenol-sensitive adenylate cyclase activity might be secondary to a decrease in the number or affinity of beta-adrenergic receptors. [^{125}I]CYP specific binding characteristics were evaluated. Scatchard plot [24] analysis of the data in Fig. 4 indicates that, while

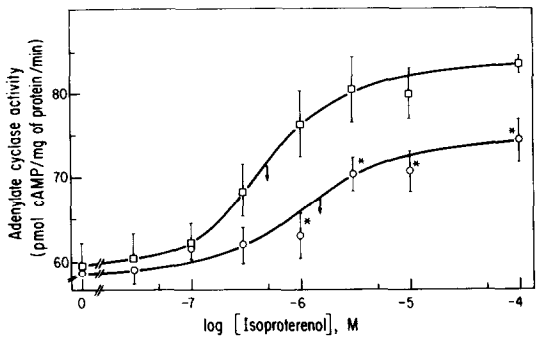

Fig. 3. Effect of sucrose feeding on isoproterenol stimulation of adenylate cyclase activity in liver cell membranes prepared from ADX rats. Adenylate cyclase activity was measured in the presence or absence of various concentrations of isoproterenol. Animals in this experiment were adrenalectomized 1 day prior to beginning the sucrose (○) or the control (□) diet. Asterisks (*) indicate values significantly lower ($P < 0.05$) than the comparable control as determined by Student's *t*-test, and the downward arrow (↓) indicates the concentration of isoproterenol producing half-maximal stimulation (EC_{50}).

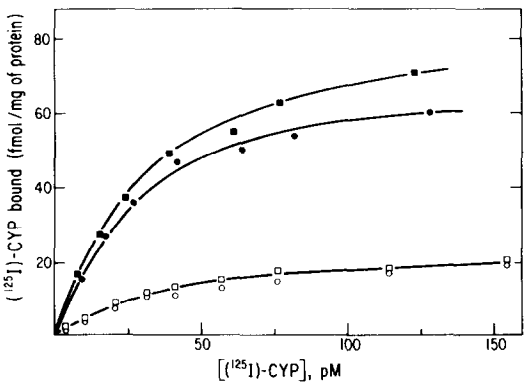

Fig. 4. Influence of adrenalectomy on [^{125}I]CYP binding to liver membranes from control and sucrose-fed rats. [^{125}I]-CYP specific binding was measured at various concentrations of [^{125}I]CYP. Each symbol represents the source of membranes from different treatment groups as follows: control diet (□), sucrose diet (○), ADX-control diet (■), and ADX-sucrose diet (●).

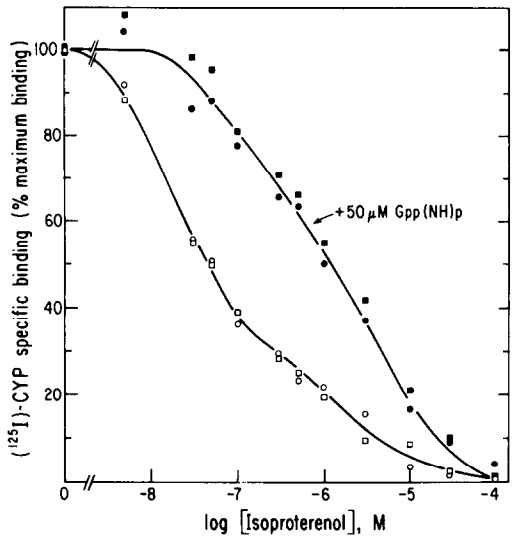

Fig. 5. Influence of Gpp(NH)p on concentration-dependent inhibition of [^{125}I]CYP specific binding by isoproterenol. Liver cell membranes prepared from ADX control-fed (□, ■) or ADX sucrose-fed (○, ●) animals were incubated with 95 pM [^{125}I]CYP in the absence or presence of various concentrations of (*l*)-isoproterenol at 37° for 60 min. The presence of the non-hydrolyzable GTP analogue Gpp(NH)p (50 μM) is indicated by closed symbols. Similar results were obtained with non-ADX liver membranes.

no significant decrease in maximal binding capacity (B_{max}) occurred in membranes from normal rats fed sucrose (i.e. B_{max} values for control-fed and sucrose-fed liver membranes were 36.9 ± 2.7 and 36.5 ± 5.9 fmoles/mg protein respectively), a small but significant decrease in [^{125}I]CYP binding capacity occurred in ADX rats fed sucrose (i.e B_{max} values decreased from 89.4 ± 1.1 to 78.4 ± 3.4 fmoles/mg protein; $P < 0.05$). None of the perturbations had significant effects on the concentration of [^{125}I]CYP giving half-maximal binding (K_d) as determined by Scatchard plot [24] analysis (i.e. K_d values range from 32.9 to 50.5 pM in repeated experiments on liver membranes from control and sucrose-fed ADX and normal rats).

In another series of experiments (Fig. 5), concentration-dependent inhibition of [^{125}I]CYP binding by isoproterenol was measured as an index of agonist affinity to β-adrenergic receptors. Sucrose feeding had no effect on the ability of isoproterenol to displace [^{125}I]CYP bound to liver plasma membranes or the ability of the non-hydrolyzable GTP analogue Gpp(NH)p to shift the β-receptors to a lower agonist affinity state (Fig. 5).

It has been proposed that β receptor-induced methylation of plasma membrane phospholipids leads to increased membrane fluidity and coupling to adenylate cyclase [25]. To ascertain whether sucrose diet-induced reductions in β-adrenergic responsiveness were associated with alterations in phospholipid methylation, the activities of phospholipid methytransferase I and methyltransferase II were determined in hepatic membranes from control and sucrose-fed rats. As illustrated in Table 3, both enzyme activities were reduced by 25% following sucrose feeding resulting in reduced synthesis of phosphatidylmonomethylethanolamine (PME), phosphatidyldimethylethanolamine (PDE) and phosphatidylcholine (PC). The addition of isoproterenol had no significant influence on either methyltransferase activity. In addition, the hepatic levels of AdoMet, which acts as the endogenous methyl donor, were reduced in sucrose-fed animals (control = 90.4 ± 4.9 and sucrose = 65.3 ± 6.5 nmoles/g tissue). AdHcy levels were unchanged (control = 11.1 ± 0.1 and sucrose = 12.4 ± 1.2 nmoles/g tissue).

DISCUSSION

The level of activity of the β-adrenergic receptor apparatus has been shown to be influenced by a number of factors including age, sex, diet and hormonal status [27]. In a previous study, we had found that hyperactivity of the sympathetic nervous system induced by a sucrose diet resulted in a significant reduction in α-adrenergic response of the rat liver [27]. The current studies examined whether or not similar changes occur in the β-adrenergic receptor system. Our results indicate that such a reduction in β-receptor responses did take place following sucrose feeding; however, its occurrence may not have been

Table 3. Effect of sucrose feeding on liver membrane phospholipid methyltransferase activity*

Group	Total activity†	Amount of phospholipid formed‡		
		PC	PDE	PME
Control PMT I	350 ± 11	70 ± 4	116 ± 6	164 ± 12
Sucrose PMT I	264 ± 14§	54 ± 3§	81 ± 12§	120 ± 6§
Control PMT II	4850 ± 450	1355 ± 81	1550 ± 156	1900 ± 212
Sucrose PMT II	3640 ± 100§	966 ± 24	1208 ± 54	1477 ± 93

* All values are the mean ± S.E.M. for duplicate determinations from two separate experiments using separate membrane preparations.

† Activity is expressed as pmoles ^{3}H-methyl transferred · (mg protein)$^{-1}$ · (30 min)$^{-1}$.

‡ Amounts are expressed as pmoles of phospholipid formed calculated by the formula of Audubert and Vance [26].

§ Indicates value significantly lower ($P < 0.05$) than control membranes.

secondary to increased activity of the sympathetic nervous system.

The metabolic effects of a sucrose-supplemented diet have received considerable attention in light of the possible implications for contemporary diets with increased sucrose components. It has been documented previously that substitution of drinking water with a 10% solution of sucrose leads to alterations in a number of hormones which directly or indirectly might influence hepatic β-adrenergic receptor activity [11]. For instance, sucrose feeding leads to a sustained increase in hepatic sympathetic tone, a 50% rise in fasting insulin levels, a 3 to 4-fold increase in plasma corticosteroid concentrations as well as changes in hepatic lipid synthesis [10, 11, 28]. These observations led us to investigate the influence of sucrose feeding on adrenergic receptor responses. Studies of α-adrenergic responses showed a 60–100% reduction in receptor-initiated events including release of intracellular Ca^{2+}, activation of $[Na^+\text{-}K^+]$-ATPase activity and glucose release [27]. Studies with animals treated with 6-OH dopamine, which produces a "chemical sympathectomy", did not yield such reductions in α-receptor responsiveness, indicating an essential role for the sympathetic nervous system in the production of this effect.

It should be noted that in the liver both α_1 and β_2 receptor activation leads to glucose mobilization and that the primary consequence resulting from the sucrose diet is an elevation of circulating plasma glucose levels. Alterations in liver metabolism might be expected to reflect either direct adaptation of the organ to elevated glucose levels or indirect influences on the organ such as that provided by increased sympathetic activity, increased circulating levels of insulin, or corticosteroids. A third possibility is that both direct and indirect adaptive mechanisms could lead to changes in receptor responsiveness.

El-Refai and Chan [4] found that fasting results in an increase in β-adrenergic responsiveness in rat liver. This intervention is associated with hypoglycemia and increased circulating catecholamine levels primarily of adrenal origin. Increased responsiveness in this circumstance is probably associated with an increased number of β-adrenergic receptors [4]. Adrenalectomy during normal access to food also results in an increase in the number of hepatic β receptors, as does an increase in thyroid hormone levels [5, 6]. El-Refai and Chan point out, however, that adrenalectomy and abnormal thyroid conditions are accompanied by altered spontaneous dietary intake and that such dietary changes may be an important determinant of adrenergic responsiveness in rat liver [4].

The central observation of our study is that the ability of maximally effective concentrations of isoproterenol to elevate adenylate cyclase activity was reduced by 50–75% in hepatic membranes prepared from sucrose-fed rats (Fig. 2, Table 2). This decreased responsiveness appears to have been due to a rather specific change in β-receptor effectiveness since there was no change in basal, GTP-, fluoride-azide- or glucagon-stimulated cyclase activities (Table 1). Based on [^{125}I]CYP binding studies, the decreased responsiveness does not appear to be attributable to decreased receptor number since no significant change in B_{max} was observed in normal or adrenalectomized animals on the sucrose diet (as compared to normal diet controls). Isoproterenol affinity was also unchanged following sucrose feeding. These findings indicate that binding events were not altered by the sucrose diet, nor was the catalytic potential for cyclase activity diminished. The most likely explanation for the diminished response would appear to be a reduction in efficiency of receptor coupling.

While the precise events constituting the coupling process are both unclear and controversial, we did examine two processes which have been hypothesized to be important intermediate steps: (1) the GTP-induced decrease in β-receptor agonist affinity; and (2) phospholipid methylation by methyltransferase enzymes.

Isoproterenol was able to displace [^{125}I]CYP with identical potency both in the absence or presence of GTP in hepatic membranes from sucrose or control diet animals. Thus, it appears that the role of the GTP-regulatory subunit in modifying receptor affinity can be fully expressed in sucrose-fed animals. These results do not exclude the possibility that the GTP-regulatory subunit does not interact effectively with the catalytic subunit.

Hirata *et al.* [25] reported that β-receptor occupancy in rat reticulocyte ghosts leads to increased membrane fluidity and increased activity of phospholipid methyltransferases. This model of fluidity-based receptor coupling is, however, not supported by other observations [29, 30]. While rapid changes in fluidity induced by β-receptor agonists may not be the mechanism leading to activation of adenylate cyclase, differences in membrane fluidity due to altered phospholipid content may modulate the efficiency of β-receptor coupling events within the context of the floating receptor hypothesis. Thus, even though isoproterenol does not affect phospholipid methylation, its ability to increase c-AMP synthesis may be impaired in a less fluid membrane environment. We found the activity of phospholipid methyltransferase I and methyltransferase II to be reduced by 25% in hepatic membranes from sucrose-fed rats under conditions of optimal activity. This would indicate that sucrose feeding may decrease the level of phospholipids whose synthesis is dependent on activity of these enzymes (e.g. phosphatidylmonomethylethanolamine and phosphatidylcholine). The decrease in enzyme activity was accompanied by a decrease in the levels of *S*-adenosyl-L-methionine which functions as the endogenous methyl donor for the methytransferases. The possible significance of these changes in reducing the efficiency of β-adrenergic coupling must await further clarification of the sequence of such events. Differences between β-adrenergic and glucagon responses may be attributable to the existence of a substantial receptor reserve in the case of the latter hormone [31].

Some information on the origin of the above changes during sucrose feeding was provided by studies on 6-OH dopamine-treated and adenalectomized animals. 6-OHDA reduced the norepinephrine content of the liver by 87%, indicating its effectiveness at destroying sympathetic nerve endings, but failed

to block the development of decreased β-receptor responsiveness (Table 2). Thus, sucrose-dependent modulation of the β-receptor system differs from that of the α-receptor system [27]. A possible role for corticosteroids was tested by comparing the influence of sucrose feeding on adrenalectomized rats versus their normal counterparts. While the reduction in β responsiveness was somewhat less than in control rats, a clear decrease was present in the sucrose-fed adrenalectomized group (Fig. 3). Therefore, the mechanism by which β-receptor response is diminished by sucrose diet in rat liver does not seem to involve the "indirect" responses of elevated sympathetic nervous system activity or increased corticosteroid levels.

In summary, substitution of a sucrose-containing solution for normal drinking water caused a significant and selective decrease in β-adrenergic responses of the rat liver. The mechanism underlying the reduction did not require intact sympathetic innervation or adrenal function, but may involve an alteration in the composition of phospholipids in the hepatic plasma membrane.

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