Biochemical Pharmacology, Vol. 45, No. 12, pp. 2441–2447, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

EFFECTS OF ADRENERGIC AGONISTS AND ANTAGONISTS ON THE METABOLISM OF [1-^{14}C]OLEIC ACID BY RAT HEPATOCYTES

JOSEPH O. OLUBADEWO* and MURRAY HEIMBERG†
Department of Pharmacology, University of Tennessee, Memphis, The Health Science Center, Memphis, TN 38163, U.S.A.

(*Received* 3 *September* 1992; *accepted* 24 *February* 1993)

Abstract—The possibility that the antihypertensive adrenoceptor antagonists (propranolol, phentolamine and metoprolol) may alter hepatic lipid metabolism was examined in freshly dispersed rat hepatocytes with [1-^{14}C]oleate. Propranolol (1.8×10^{-4} M) and phentolamine (1.4×10^{-4} M) increased incorporation of [1-^{14}C]oleate into cholesteryl esters by 51 and 92%, respectively, and decreased ketogenesis by 46 and 62%, respectively. While neither drug affected incorporation into total phospholipid, propranolol decreased triglyceride synthesis by 37%. These effects of propranolol and phentolamine may not occur through β- or α-receptor inhibition since neither epinephrine nor norepinephrine reversed the effects of the adrenoceptor antagonists. Although epinephrine and norepinephrine *per se* did not alter the incorporation of [1-^{14}C]oleate into triglyceride, phospholipid, cholesteryl esters or ketone bodies, they stimulated the production of $^{14}CO_2$ (control 5.6 ± 1.3; epinephrine 7.6 ± 1.1; norepinephrine 9.1 ± 0.2 nmol oleate incorporated/mg protein), and these effects were reversed by phentolamine and propanolol. The data suggest that adrenoceptor antagonists exert direct effects on hepatic metabolism of oleate.

Catecholamines are commonly regarded as catabolic hormones enhancing oxidation of free fatty acid (FFA‡) [1, 2]. Heimberg *et al.* [3] reported that epinephrine and norepinephrine decrease hepatic uptake of FFA and the output of triglyceride by the isolated perfused rat liver; ketogenesis and output of glucose by these livers are stimulated moderately. In a more recent study with isolated perfused livers from fed rats, Reinhart *et al.* [4] demonstrated that phenylephrine, a specific α-receptor agonist, induces an increase of ketogenesis via Ca^{2+}-independent mechanisms; phenylephrine also enhances oxygen uptake and the output of glucose and lactate-plus-pyruvate [4, 5]. Furthermore, it has been well established that infusion of epinephrine or norepinephrine into dogs [2, 6, 7] or humans [1, 2, 8–11] increases plasma concentrations of FFA and ketone bodies. In contrast to earlier *in vitro* [3–5] and *in vivo* [6–11] studies, Sugden *et al.* [12] reported that injection of epinephrine into 24-hr fasted rats decreases concentrations of ketone bodies in blood by 69%, but in agreement with other investigators, reported an increase in glucose and lactate concentrations. Bahnsen and Alberti [13] confirmed the observations of Sugden *et al.* [12].

There are few reports designed specifically to examine the direct effects of epinephrine and norepinephrine on hepatic metabolism of FFA. However, a potential role of adrenergic mechanisms on fatty acid metabolism is suggested by recent clinical reports that failure of antihypertensive therapy with β-adrenoceptor blockers to reduce the incidence of coronary heart disease is associated with a tendency of certain of these agents to elevate atherogenic lipoproteins during chronic administration [14–23]. Specifically, propranolol was reported to increase very low density lipoprotein (VLDL)-triglyceride and low density lipoprotein (LDL)-cholesterol, while decreasing high density lipoprotein (HDL)-cholesterol [14–18]; similar changes were reported for metoprolol [19–22]. The changes in plasma lipids induced by propranolol and metoprolol are thought to increase the risk of development of atherosclerotic cardiovascular disease [24–26]. The mechanisms by which β-blockers produce these lipid alterations have not been fully elucidated, nor have studies been done to delineate whether the effects were exerted on liver or adipose tissue or other body systems.

Hence, the objective of the present study with isolated hepatocytes was to examine the extent to which the effects of catecholamine agonists and their antagonists are exerted directly on the liver. Our conclusions were that, although epinephrine or norepinephrine produced no consistent alterations of fatty acid oxidation or esterification, propranolol and phentolamine inhibited ketogenesis and increased synthesis of cholesteryl esters, but had essentially no effect on triglyceride synthesis.

METHODS

Animals

Male Sprague–Dawley rats, weighing about 300 g

* Present address: Laboratories of Pharmacology and Toxicology, Division of Basic Pharmaceutical Sciences, College of Pharmacy, Xavier University of Louisiana, New Orleans, LA 70125.

† Corresponding author: Dr. Murray Heimberg, Department of Pharmacology, University of Tennessee, 874 Union Ave., Memphis, TN 38163. Tel. (901) 528-6000; FAX (901) 528-7300.

‡ Abbreviations: FFA, free fatty acid; HDL, high density lipoprotein; LDL, low density lipoprotein, VLDL, very low density lipoprotein; and BSA, bovine serum albumin.

(Harlan Industries, Indianapolis, IN), were fed *ad lib.* under a 12–12 hr light–darkness cycle in our animal housing facility. As required, fasted rats were deprived of food, but allowed free access to water 24 hr prior to the experiment.

Chemicals

[1-^{14}C]Oleic acid (free acid) was purchased from the NEN-Du Pont Co., Boston, MA. Water-soluble salts of adrenergic agonists and antagonists used in the present study were: epinephrine bitartrate, norepinephrine hydrochloride, DL-propranolol hydrochloride, phentolamine hydrochloride and metoprolol tartrate. These adrenergic agents were purchased from the Sigma Chemical Co., St. Louis, MO. Hyamine hydroxide was purchased from the Research Products International Corp., Mt. Prospect, IL. Oleic acid (99% purity) was obtained from Nu-Chek Prep., Elysian, MN, while bovine serum albumin (BSA, Fraction V, powder) was obtained from the Sigma Chemical Co. or Miles Laboratories Inc., Elkhart, IN. The albumin was defatted by a procedure routinely used in our laboratory [27]. All other chemicals were of reagent grade.

Preparation of hepatocytes

Cell-dispersion buffer: Ca^{2+}-free Krebs–Henseleit bicarbonate buffer (60 mL) containing 300 mg glucose/dL and 50 mg collagenase/dL (Worthington Biochemical Products, Freehold, NJ), pH 7.4, gassed continuously with 95% O_2–5% CO_2.

Cell-suspension buffer: Ca^{2+}-free Krebs–Henseleit bicarbonate buffer containing 300 mg glucose/dL (for initial three washes) and 100 mg glucose/dL (for final suspension of washed hepatocytes).

Hepatocytes were prepared by a modification of the method of Berry and Friend [28]. Livers were isolated surgically and then perfused with the cell-dispersion buffer in a recycling system, as previously described [3, 29]. The collagenase-treated liver was passed through a nylon mesh filter. Hepatocytes were washed by a series of low speed centrifugations (672 *g*), followed by resuspension of the pellet with the cell-suspension buffer. Viability of hepatocytes was estimated by exclusion of trypan blue, and preparations with less than 90% dye exclusion were discarded. The protein content of tissue in the cell suspension was measured as described by Markwell *et al.* [30].

Preparation of oleate–albumin complex

An aliquot of ethanolic solution of unlabeled oleic acid containing 200 μmol was mixed with 5.4 μCi of [1-^{14}C]oleic acid and titrated to a phenolphthalein end-point with 0.1 M NaOH. The ethanol was briskly evaporated on a hot-plate, and the dried "soap" was dissolved on a warm hot-plate with approximately 5 mL of 0.9% NaCl solution. The warm "soap" solution was treated with a solution (pH 7.4) of fatty acid-free BSA to give a molar ratio of 1:3 of albumin:oleate complex. The working solution was made up to 10 mL.

Incubation of hepatocytes

Cells were incubated for 40 min at 37° in 25-mL Erhlenmeyer flasks in a Dubnoff metabolic shaker (approximately 80 cycles/min). Each flask was oxygenated with 95% O_2–5% CO_2 and then stoppered before it was incubated. The control incubation medium was Krebs–Henseleit bicarbonate buffer containing final concentrations of 2.75 mM glucose, 2.08 mM Ca^{2+}, and 1.0 mM [1-^{14}C]oleate as a complex with fatty acid-free bovine serum albumin (about 3% albumin). The final specific activity of the oleate was 60,000 dpm/μmol (approximately 240,000 dpm/incubation vessel). The experimental incubation medium contained, in addition, final concentrations of either epinephrine or norepinephrine (2.7×10^{-7} to 1.6×10^{-3} M), phentolamine (2.4×10^{-6} to 1.3×10^{-4} M), propranolol (2.5×10^{-6} to 1.3×10^{-4} M), metoprolol (1.8×10^{-6} to 1.3×10^{-4} M), or a combination of agonist with antagonist. As pointed out under "Chemicals", these adrenergic agents were water-soluble, so aqueous solutions of them were used. For certain experiments, as indicated, Ca^{2+} was omitted from the media. Two milliliters of cell suspension was incubated with 2.0 mL of the control or experimental media. Non-specific, zero-time incorporation of radioactivity was measured with cells that had not been incubated at 37°, but were mixed at room temperature for about 10 sec with the incubation media described above. Incorporation into lipids and ketone bodies was subtracted from that measured after 40 min of incubation.

At the end of incubation period, a 2.0-mL aliquot of the cell suspension (cells plus incubation medium) was removed for extraction of lipids [29, 31] and the remaining aliquot was deproteinized with 0.5 mL of 15% $HClO_4$. The extracted lipid was separated by TLC [29] and the bands corresponding to FFA, phospholipids, cholesteryl esters, and triglycerides were scraped into scintillation vials. Scintillation fluor (10.0 mL Biocount; Research Products International Corp.) was added to each sample, and radioactivity was counted in a liquid scintillation spectrometer with a data reduction package (Beckman LS 7500). The perchloric acid-deproteinized aliquots were neutralized to a methyl orange end-point with 10% KOH. After centrifugation, a 0.1-mL aliquot of the supernatant was treated with Biocount, and radioactivity was measured by liquid scintillation spectrometry. The radioactivity in the neutralized acid-soluble extracts represents primarily ketone bodies produced by the hepatocytes, as reported by Stakkestaad and Bremer [32]. Statistical analyses of differences due to the presence of drug were computed by Student's *t*-test.

Measurement of $^{14}CO_2$ production

In this set of experiments $^{14}CO_2$ production was measured as an additional index of fatty acid oxidation. Erhlenmeyer flasks with central wells were used. The hepatocytes were incubated in the outer well, while the inner well contained hyamine hydroxide with a filter paper wick in it. The incubation was terminated by adding 1.0 mL of 4.0 N HCl into the outer well. The acidified cell suspension was shaken for an additional 1 hr to ensure complete displacement of $^{14}CO_2$. Hyamine chloride plus the filter paper wick were transferred into the scintillation

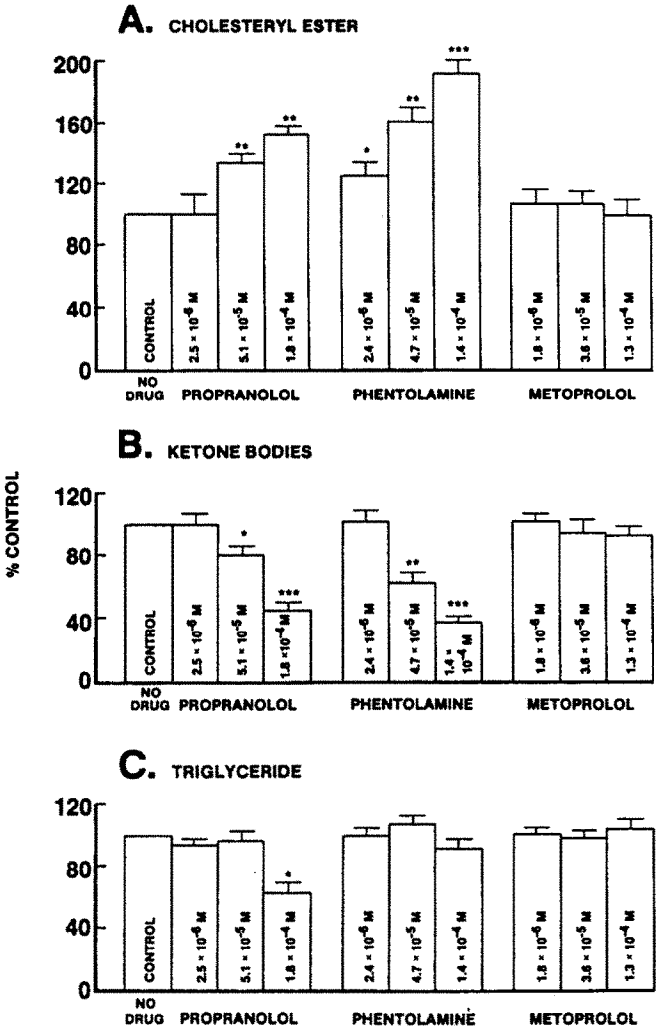

Fig. 1. Effects of propranolol, phentolamine and metoprolol on the incorporation of [1-^{14}C]oleate into cholesteryl esters (A), ketone bodies (B) and triglyceride (C). Hepatocytes were incubated in a total volume of 4.0 mL containing BSA as a complex with 1.0 mM [1-^{14}C]oleate in Krebs–Henseleit bicarbonate buffer, and either no drug (control), or propranolol (2.5 to 180 μM), phentolamine (2.4 to 140 μM) or metoprolol (1.8 to 130 μM). Cholesteryl esters and triglyceride were measured from corresponding bands following separation by TLC. Conversion of oleate to ketone bodies corresponded to the radioactivity of neutralized acid-soluble extracts. Statistical significance of differences between drug effects and control: *$P < 0.05$; **$P < 0.005$; and ***$P < 0.001$. Values are means ± SEM of four or more separate preparations of hepatocytes from fed rats.

vial, treated with scintillation fluor and counted in a liquid scintillation spectrometer.

RESULTS

Effects of propranolol, metoprolol and phentolamine on oleate metabolism

Propranolol and phentolamine, but not metoprolol had significant effects on the metabolism of [1-^{14}C]-oleate by hepatocytes from fed rats (Fig. 1). Metoprolol (1.8×10^{-6} to 1.3×10^{-4} M) did not alter the incorporation of [1-^{14}C]oleate into triglyceride, cholesteryl esters, or ketone bodies. At 2.5×10^{-6} M, neither propranolol nor phentolamine affected the incorporation of [1-^{14}C]oleate into triglyceride or ketone bodies, although 2.4×10^{-6} M phentolamine increased incorporation into cholesteryl ester by 24% ($P < 0.05$). At higher concentrations, both propranolol (5.1×10^{-5} M, 1.8×10^{-4} M) and phentolamine (4.7×10^{-5} M, 1.4×10^{-4} M) had similar effects on incorporation into ketone bodies and cholesteryl esters, but phentolamine was more potent, i.e. these drugs increased incorporation of [1-^{14}C]oleate into cholesteryl esters (51% increase with the highest concentration of propranolol; 92% with phentolamine, Fig. 1A; Table 1), but decreased incorporation into ketone bodies (54% of control with the highest concentration of propranolol; 38%

Table 1. Effects of catecholamine agonists and antagonists on esterification of [1-^{14}C]oleate by hepatocytes from fed rats

Drug added	Concn of drug (μM)	Esterification products (nmol oleate incorporated/mg protein/40 min) PL	TG	CE
None		5.66 ± 1.27	23.50 ± 4.30	0.69 ± 0.07
EPI	55	5.81 ± 1.50	22.86 ± 3.79	0.78 ± 0.11
NE	55	6.61 ± 1.56	22.71 ± 3.20	0.75 ± 0.06
Propranolol	187	6.17 ± 1.18	15.40 ± 1.87*	0.98 ± 0.05*
Phentolamine	133	5.81 ± 0.69	20.52 ± 3.13	1.40 ± 0.09†
Phentolamine +EPI	133 55	5.38 ± 1.13	22.35 ± 3.11	1.42 ± 0.04†
Propranolol +EPI	187 55	6.98 ± 0.98	18.22 ± 2.16	1.02 ± 0.06†
Phentolamine +NE	133 55	4.75 ± 0.72	20.97 ± 2.57	1.22 ± 0.04†
Propranolol +NE	187 55	7.69 ± 1.12	20.08 ± 1.24	1.08 ± 0.07†

Hepatocytes were incubated for 40 min in a total volume of 4.0 mL containing BSA complex with 1.0 mM [1-^{14}C]oleate in Krebs–Henseleit bicarbonate buffer, and either no drug, or 55 μM EPI, 55 μM NE, 187 μM propranolol, 133 μM phentolamine or a combination of agonist plus antagonist. Esterification products were isolated from (cells + medium) and separated by TLC as described in the text. Each value is the mean ± SEM of four separate hepatocyte preparations. Abbreviations: PL, phospholipid; TG, triglyceride; CE, cholesteryl esters; EPI, epinephrine; and NE, norepinephrine.

*,† Statistically significant compared to control; *$P < 0.05$, and †$P < 0.005$.

with phentolamine; Fig. 1B; Table 2). Triglyceride synthesis was perturbed only by propranolol (1.8×10^{-4} M), being reduced to 63% of control (Fig. 1C; Table 1).

The omission of Ca^{2+} from the incubation medium depressed incorporation into ketone bodies, triglyceride, cholesteryl esters and total phospholipid (Table 3). However, the omission of Ca^{2+} did not alter the relative effects of these drugs observed in the Ca^{2+}-containing control incubation medium.

Interaction with catecholamine agonists

The effects of propranolol and phentolamine on hepatic lipid metabolism were assumed to be produced via their respective adrenergic receptors. This was examined by incubating the hepatocytes with norepinephrine or epinephrine. In preliminary experiments, it was established that the incorporation of [1-^{14}C]oleate into triglyceride or into acid-soluble products by hepatocytes from fed and fasted rats was linear for up to 50 min. Studies with fasted rats are not reported here because neither epinephrine nor norepinephrine altered this linearity, and concentrations of 2.7×10^{-7} to 5.5×10^{-4} M of either norepinephrine or epinephrine had no significant effects on incorporation of [1-^{14}C]-oleate into triglyceride, cholesteryl esters, total phospholipid or ketone bodies by hepatocytes from fasted rats. Moreover, norepinephrine and epinephrine (5.5×10^{-5} M) did not alter metabolism of [1-^{14}C]oleate, by hepatocytes from fed rats, and did not modify any of the effects of phentolamine or propranolol (Tables 1 and 2).

In a few cases, production of $^{14}CO_2$ by hepatocytes from fed rats was measured (Table 2). Epinephrine

Table 2. Effects of catecholamine agonists and antagonists on oxidation of [1-^{14}C]oleate by hepatocytes

Drug added	Concn of drug (μM)	Oxidation products (nmol oleate incorporated/mg protein) $^{14}CO_2$	Ketone bodies
None		5.56 ± 1.34	19.41 ± 2.00
EPI	55	7.60 ± 1.08*	17.42 ± 1.51
NE	55	9.05 ± 0.17†	17.43 ± 1.74
Propranolol	187	5.31 ± 0.98	7.78 ± 0.72†
Phentolamine	133	6.04 ± 0.75	7.08 ± 0.91†
Propanolol +EPI	187 55	5.82 ± 0.95	9.95 ± 0.95†
Phentolamine +EPI	133 55	5.10 ± 0.71	7.49 ± 1.25†
Propranolol +NE	187 55	5.74 ± 1.14	9.62 ± 1.13†
Phentolamine +NE	133 55	5.64 ± 1.40	7.99 ± 1.18†

Hepatocyte preparations and incubation conditions were the same as in the legend to Table 1. At the end of the incubation period, the cell suspension was acidified with 4.0 N HCl to displace $^{14}CO_2$ into hyamine hydroxide inside the central well of the incubation flask. The acidified cell suspension was shaken for 1 hr to ensure complete displacement of $^{14}CO_2$. The radioactive content of the neutralized acid-soluble extract was used to compute the rate of ketone production. Values are means ± SE of four separate hepatocyte preparations. See Table 1 for definitions of abbreviations.

*,† Significant difference between drug effects and control: *$0.05 < P < 0.1$, and †$P < 0.005$.

Table 3. Effects of catecholamine antagonists on metabolism of [1-^{14}C]oleate by hepatocytes from fed rats incubated in Ca^{2+}-free buffer

Drug added	Drug concn (μM)	(nmol Oleate incorporated/mg protein/40 min)			
		PL	TG	CE	Ketone bodies
None (Ca^{2+}-free medium)		4.57 ± 0.80 (100)	19.76 ± 1.77 (100)	0.66 ± 0.12 (100)	13.16 ± 1.19 (100)
Propranolol	51	4.41 ± 0.59 (100 ± 14)	17.12 ± 1.50 (87 ± 2)	0.79 ± 0.13* (124 ± 8)	9.24 ± 0.66† (71 ± 3)
Phentolamine	47	3.78 ± 0.46 (89 ± 15)	18.71 ± 1.83 (95 ± 2)	0.98 ± 0.12‡ (158 ± 14)	7.33 ± 90.49† (57 ± 4)
Metoprolol	36	3.94 ± 0.28 (97 ± 14)	18.11 ± 1.24 (93 ± 4)	0.62 ± 0.11 (98 ± 4)	11.44 ± 0.99 (87 ± 4)
None (normal incubation) medium		5.84 ± 0.88 (131 ± 15)	31.52 ± 2.76 (161 ± 7)	0.87 ± 0.11 (126 ± 6)	16.07 ± 1.34 (123 ± 4)

Ca^{2+}-free medium was prepared by omission of Ca^{2+}. Incubation of hepatocytes and assay of metabolites were the same as described in the legends to Tables 1 and 2. The bottom line was inserted to compare oleate metabolism in Ca^{2+}-free medium with that in the normal Ca^{2+}-containing medium. Values are means ± SEM of four to six separate hepatocyte preparations. Values in brackets are percent of control incorporation in the Ca^{2+}-free incubation medium. See Table 1 for definitions of abbreviations.

*–‡ Significant difference between drug effect and absence of drug: $*0.05 < P < 0.01$, $\dagger P < 0.005$, and $\ddagger P < 0.05$.

and norepinephrine (each 5.5×10^{-5} M) enhanced incorporation of [1-^{14}C]oleate into $^{14}CO_4$ (control: 5.6 ± 1.;3; epinephrine: 7.6 ± 1.1; norepinephrine: 9.0 ± 0.2 nmol oleate incorporated/mg protein/40 min). Propranolol (1.8×10^{-4} M) and phentolamine (1.3×10^{-4} M) did not alter basal $^{14}CO_2$ generation, but reduced to control level the increased production by epinephrine and norepinephrine.

DISCUSSION

In the present study, metoprolol differed from propranolol and did not alter the incorporation of [1-^{14}C]oleate into triglyceride, phospholipid, cholesteryl ester and ketone bodies. These data suggested that the lipid-elevating effect of metoprolol *in vivo* [19, 20] may not be due to a direct effect on the liver. The similarity of the modification of hepatocyte metabolism of FFA by propranolol and phentolamine was rather unexpected since the two drugs interact at different adrenergic receptors. The use of propranolol (a nonselective β-blocker) to treat hypertension in humans has been associated with elevation of plasma triglyceride and LDL-cholesterol while lowering HDL-cholesterol, thus contributing to increased risk of ischemic heart disease [14–18]. Phentolamine has not been associated with alteration of lipoprotein levels, perhaps because phentolamine has not usually been used on a chronic basis. The effects of propranolol on plasma lipoproteins are also shared by sotalol, another non-specific β-blocker [33] and metoprolol, a cardio-selective β-adrenoceptor antagonist [19, 20], although Pasotti *et al.* [34] reported that administration of metoprolol for 12 weeks does not alter plasma concentrations of lipids and lipoproteins. The lack of effect of metoprolol observed in this study differed from the results of Bell [35] who reported that metoprolol increases incorporation of [1-^{14}C]oleate into phospholipids and diglycerides by 20–50%; however, in agreement with our data, incorporation into triglyceride and cholesteryl ester was unaltered. The difference might be attributed to the fact that Bell [35] used rat liver minces and a higher concentration of metoprolol (1×10^{-3} M), in contrast to our use of hepatocytes and a lower range (1.8×10^{-6} to 1.3×10^{-4} M) of metoprolol concentrations.

The most prominent effects of propranolol and phentolamine were on the ketogenesis and synthesis of cholesteryl esters. The enhanced synthesis of cholesteryl esters was consistent with a stimulation of hepatic acyl-CoA:cholesterol acyltransferase (ACAT: EC 2.3.1.26) by these drugs. If this were the case, hepatic ACAT may be different from the arterial enzyme since the arterial ACAT is inhibited by high concentrations (1.0 mM) of propranolol [36]. Alternatively, it is possible that these drugs inhibited a cholesteryl ester hydrolase. The elevation of cholesteryl esters by propranolol suggests a possible mechanism by which LDL-cholesterol concentration may be elevated by chronic propranolol [14–18, 21, 22, 37]. One may presume increased secretion of CE in the VLDL, followed by metabolism to form LDL. Although phentolamine is not commonly used chronically to treat hypertension, prazosin, another α-adrenoceptor antagonist is, and prazosin has been reported to either lower plasma cholesterol and triglyceride [14, 38], or have no effect [30] in hypertensive patients. It is expected that the effects of phentolamine ($\alpha_1 + \alpha_2$ antagonist) would be similar to those of prazosin (α_1 antagonist). As shown in the present study, phentolamine increased cholesteryl ester synthesis, in agreement with effects of prazosin reported by Bell [35]. The pronounced effect of phentolamine or that reported for prazosin

does not readily provide an explanation into the mechanism by which therapeutic doses of prazosin affect plasma lipids in hypertensive patients [14, 38, 39].

The direct antiketogenic effects of propranolol and phentolamine have not been reported previously. In a recent review, Bahnsen *et al.* [40] concluded that in humans, epinephrine- and norepinephrine-induced increases in ketogenesis are secondary to increases in non-esterified fatty acid substrate supply, resulting from stimulation of lipolysis. In the rats, however, there are confusing data. Thus, norepinephrine and epinephrine have been shown to either decrease [12, 41], increase [3–5, 42, 43] or have no effect on ketogenesis [44].

The report by Sugden *et al.* [44] showed that norepinephrine and epinephrine do not alter ketogenesis but increase CO_2 production when hepatocytes from fed and fasted rats are incubated with [1-^{14}C]oleate. In studies by other investigators, the effects of catecholamine antagonists were not reported, except in the study by Heimberg *et al.* [3] where dibenzyline was shown to produce a slight increase in ketone body output. In our present study, phentolamine and propranolol strongly inhibited ketogenesis: the inhibition was dose dependent. The anti-ketogenic effects of propranolol and phentolamine do not seem to be related to their receptor properties, because effective concentrations of the antagonists were much higher than those encountered in plasma during therapeutic administration. Additionally, neither epinephrine nor norepinephrine exerted any significant effects of their own on the hepatocyte and they did not alter effects of propranolol or phentolamine on incorporation of [1-^{14}C]oleate into acid-soluble extracts. The lack of consistency in the effects of catecholamine agonists on ketogenesis provided no clue for elucidating the mechanism(s) by which propranolol or phentolamine might have exerted their antiketogenic effects in the present study. Nevertheless, it can be speculated that antiketogenic effects might be exerted either through inhibition of uptake of free fatty acid by hepatocytes, or diversion of free fatty acid from oxidative into esterification pathways. However, this is unlikely since propranolol and phentolamine did not increase the amount of [1-^{14}C]oleate remaining at the termination of incubation, nor was there an increased synthesis of triglyceride and phospholipids, and the increased incorporation into cholesteryl esters (0.3 to 0.7 nmol oleate/mg protein) was nowhere near the decrease of ketogenesis by approximately 12 nmol oleate/mg protein. Nevertheless, the reported *in vivo* elevation of plasma triglyceride [14–18] by propranolol may be due, in part, to its anti-ketogenic effect because, in many cases, inhibition of the ketogenic pathway is commonly accompanied by diversion of free fatty acid to the esterification pathway [31, 45].

In conclusion, the concentrations of phentolamine and propranolol required for eliciting effects *in vitro* on hepatic lipid metabolism are much higher than those achieved during therapeutic administration, and do not lend themselves readily to interpretation with respect to mechanisms by which catecholamine antagonists alter plasma lipid profiles during management of hypertension. Nevertheless, the increased synthesis of cholesteryl esters may suggest a probable method by which propranolol increases the LDL-cholesterol content during chronic administration. The antiketogenic effects of propranolol and phentolamine probably deserve further investigation to establish a clinical significance for such an effect.

Acknowledgements—The authors wish to thank Kathleen Ryan and Richard Smith for their excellent technical assistance and also Rita Sparks, for secretarial assistance. This research was supported by Grant HL-27850 from the National Institutes of Health, U.S. Public Health Service.

REFERENCES

1. Schade DS and Eaton RP, The regulation of plasma ketone body concentration by counterregulatory hormones in man. *Diabetes* **28**: 5–10, 1979.
2. Basso LU and Havel RJ, Hepatic metabolism of free fatty acids in normal and diabetic dogs. *J Clin Invest* **49**: 537–547, 1970.
3. Heimberg M, Fizette NB and Klausner H, The action of adrenal hormones on hepatic transport of triglycerides and fatty acids. *J Am Oil Chem Soc* **41**: 774–779, 1964.
4. Reinhart PH, Taylor WM and Bygrave FL. The contribution of both extracellular and intracellular calcium to the action of α-adrenergic agonists in perfused rat liver. *Biochem J* **220**: 35–42, 1984.
5. Taylor WM, Reinhart PH and Bygrave FL, Stimulation by α-adrenergic agonists of Ca^{2+} fluxes, mitochondrial oxidation and gluconeogenesis in perfused rat liver. *Biochem J* **212**: 555–565, 1983.
6. Gray DE, Lickley HLA and Vranic M, Physiologic effects of epinephrine on glucose turnover and plasma free fatty acid concentrations mediated independently of glucagon. *Diabetes* **29**: 600–608, 1980.
7. Steiner KE, Fuchs H, Williams PE, Stevenson RW, Cherrington AD and Alberti KGMM, Regulation of ketogenesis by epinephrine and norepinephrine in the overnight-fasted, conscious dog. *Diabetes* **34**: 425–432, 1985.
8. Christensen MJ, Alberti KGMM and Brandsborg O, Plasma catecholamines and blood substrate concentrations: Studies in insulin-induced hypoglycemia and after adrenaline infusions. *Eur J Clin Invest* **5**: 415–423, 1975.
9. Clutter WE, Bier DM, Shah SD and Cryer PE, Epinephrine plasma metabolic clearance rates and physiologic thresholds for metabolic and hemodynamic actions in man. *J Clin Invest* **66**: 94–101, 1980.
10. Pernet A, Walker M, Gill GV, Orskov H, Alberti KGMM and Johnston DG, Metabolic effects of adrenaline and noradrenaline in man: Studies with somatostatin. *Diabete Metab* **10**: 98–105, 1984.
11. Silverberg AB, Shah SD, Haymond MW and Cryer PE, Norepinephrine: Hormone and neurotransmitter in man. *Am J Physiol* **234**: E252–E256, 1978.
12. Sugden MC, Watts DI and Marshall CE, Effects of adrenaline on ketogenesis from long- and medium-chain fatty acids in starved rats. *Biochem J* **204**: 749–756, 1982.
13. Bahnsen M and Alberti KGMM, Epinephrine inhibits fasting ketogenesis in the rat *in vivo*. *Diabetologia* **25**: 138, 1983.
14. Leren P, Eide I, Foss OP, Helgeland A, Hjermann I, Holme I, Kjeldsen SE and Lund-Larsen PG, Antihypertensive drugs and blood lipids: The Oslo study. *Br J Clin Pharmacol* **13**: 441S–444S, 1982.
15. Tanaka N, Sokaguchi K, Oshige T, Niimura T and Kenehisa T, Effects of chronic administration of

propranolol on lipoprotein composition. *Metabolism* **25**: 1071–1075, 1976.
16. Strej D and Mymin D, Effects of propranolol on HDL cholesterol concentrations. *Br Med J* **2**: 1495, 1978.
17. Day JL, Simpson N, Metcalfe J and Page RL, Metabolic conseqences of atenolol and propranolol in the treatment of essential hypertension. *Br Med J* **1**: 77–80, 1979.
18. Leon AS, Agre J, McNally C, Bell C, Neibling M, Grimm R and Hunninghake DB, Blood lipid effects of antihypertensive therapy: A double-blind comparison of the effects of methyldopa and propranolol. *J Clin Pharmacol* **24**: 209–217, 1984.
19. Rossner S and Weiner L, A comparison of the effect of atenolol and metoprolol on serum lipoproteins. *Drugs* **25** (Suppl 2): 322–325, 1983.
20. Shaw J, England JDF and Hua ASP, Beta-blockers and plasma triglycerides. *Br Med J* **1**: 986, 1978.
21. Lacourciere Y, Poirier L, Boucher S and Spenard J, Comparative effects of diltiazem sustained release formulation and metoprolol on ambulatory blood pressure and plasma lipoproteins. *Clin Pharmacol Ther* **48**: 318–324, 1990.
22. Kannel WB and Carter BL, Initial drug therapy for hypertensive patients with hyperlipidemia. *Am Heart J* **118**: 1012–1021, 1989.
23. Canadian Consensus Conference on Cholesterol, The prevention of heart and vascular diseases by altering serum cholesterol and lipoprotein risk factors. *Can Med Assoc J* **139** (Suppl): 1–8, 1988.
24. Miller NE, Coronary aterosclerosis and plasma lipoproteins: Epidemiology and pathophysiologic considerations. *J Cardiovasc Pharmacol* **4** (Suppl 2): 5190–5195, 1982.
25. Pooling Project Research Group, Relationship of blood pressure, serum cholesterol, smoking habit, relative weight, and ECG abnormalities to incidence of major coronary events. Final report of the Pooling Project. *J Chron Dis* **31**: 201–306, 1978.
26. Carlson LA, Bottinger LE and Ahfeldt PE, Risk factors for myocardial infarction in the Stockholm prospective study. *Acta Med Scand* **206**: 351–360, 1979.
27. Wilcox HG, Dunn GD and Heimberg M, Effects of several common long chain fatty acids on the properties and lipid composition of the very low density lipoprotein secreted by the perfused liver. *Biochim Biophys Acta* **298**: 39–54, 1975.
28. Berry MN and Friend DS, High-yield preparation of isolated rat liver parenchymal cells: A biochemical and fine structural study. *J Cell Biol* **43**: 506–520, 1969.
29. Soler-Argilaga C and Heimberg M, Comparison of metabolism of free fatty acid by isolated perfused livers from male and female rats. *J Lipid Res* **17**: 605–615, 1976.
30. Markwell MAK, Haas SM, Bieber LL and Tolbert NE, A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal Biochem* **87**: 206–210, 1978.
31. Folch J, Lees M and Sloane-Stanley GH, A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **226**: 497–509, 1957.
32. Stakkestaad JA and Bremer J, The metabolism of fatty acids in hepatocytes isolated from triiodothyronine-treated rats. *Biochim Biophys Acta* **711**: 90–100, 1982.
33. Lehtonen A and Viikari J, Long-term effect of sotalol on plasma lipids. *Clin Sci* **57**: 405–407, 1979.
34. Pasotti C, Capra A, Fiorellas G, Vibelli C and Chierichetti SM, Effects of pindolol and metoprolol on plasma lipids and lipoproteins. *Br J Clin Pharmacol* **13** (Suppl 2): 4355–4395, 1982.
35. Bell F, Effects of various antihypertensive agents on lipid metabolism: Alterations in the pattern of lipids synthesized from [^{14}C]oleate in rat liver *in vivo*. *J Cardiovasc Pharmacol* **7**: 443–448, 1985.
36. Bell F, Effects of antihypertensive agents propranolol, metoprolol, nadolol, prazosin, and chlorthalidone on CAT activity in rabbit and rat aortas and on LCAT activity in human plasma *in vitro*. *J Cardiovasc Pharmacol* **7**: 437–442, 1985.
37. Chatelain P, Laruel R, Vic P and Brotelle R, Differential effects of amiodarone and propranolol on lipid dynamic and enzymatic activities in cardiac sarcolemmal membranes. *Biochem Pharmacol* **38**: 1231–1239, 1989.
38. Leren P, Foss PO, Helgeland A, Hjermann I, Holme I and Lund-Larsen PG, Effect of propranolol and prazosin on blood lipids. The Oslo study. *Lancet* **2**: 4–6, 1980.
39. Cambien F and Plouin PF, Prazosin does not alter levels of plasma lipids, glucose and insulin. *J Cardiovasc Pharmacol* **7**: 516–519, 1985.
40. Bahnsen M, Burrin JM, Johnston DG, Pernet A, Walker M and Alberti KGMM, Mechanisms of catecholamine effects on ketogenesis. *Am J Physiol* **247**: E173–E180, 1984.
41. Johnston DG and Alberti KGMM, Hormonal control of ketone body metabolism in the normal and diabetic state. *Clin Endocrinol Metab* **11**: 329–361, 1982.
42. Exton JH, Friedmann N, Wong EHA, Brineawa JP, Corbin JD and Park CR, Interaction of glucocorticoids with glucagon and epinephrine in the control of gluconeogenesis and glycogenolysis in liver and of lipolysis in adipose tissue. *J Biol Chem* **247**: 3579–3588, 1972.
43. Cole RA and Margolis S, Stimulation of ketogenesis by dibutyryl cyclic AMP in isolated rat hepatocytes. *Endocrinology* **194**: 1391–1396, 1974.
44. Sugden MC, Tordoff AFC, Ilic V and Williamson DH, α-Adrenergic stimulation of [1-^{14}C]oleate oxidation to $^{14}CO_2$ in isolated rat hepatocytes. *FEBS Lett* **120**: 80–84, 1980.
45. Olubadewo J, Wilcox HG and Heimberg M, Modulation by glycerol of hepatic glycero-3-phosphate concentration, ketogenesis, and output of triglyceride and glucose in perfused livers from hyperthyroid and euthyroid rats. *J Biol Chem* **259**: 8857–8862, 1984.