Effect of Norepinephrine on Ketogenesis, Fatty Acid Oxidation, and Esterification in Isolated Rat Hepatocytes

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SUMMARY

Recent studies in man demonstrated a marked ketogenic effect of increased plasma norepinephrine concentrations as observed in diabetic ketoacidosis. Since this effect may have been due either to increased substrate supply for ketogenesis (lipolysis) or to direct hepatic activation of ketogenesis, the latter mechanism was examined in isolated rat hepatocytes. Incubation of hepatocytes with norepinephrine (10^{-7} to 10^{-4} M) resulted in a dose-dependent increase in conversion of the long-chain fatty acid [1-^{14}C]palmitate into ketone bodies and CO_2. Norepinephrine decreased [1-^{14}C]palmitate conversion into triglycerides without affecting fatty acid uptake.

Norepinephrine enhanced ketogenesis from [1-^{14}C]palmitate in a physiologic range of fatty acid concentrations (0.5–2.5 mM), but failed to affect fatty acid esterification to phospholipids or mono- and diglycerides. In contrast to long-chain fatty acids, oxidation of the medium-chain fatty acid [1-^{14}C]octanoate to ketone bodies was not enhanced by norepinephrine, whereas CO_2 production increased. The effect of norepinephrine on [1-^{14}C]fatty acid oxidation was blocked by the α_1 receptor blocker prazosine. The results demonstrate that norepinephrine diverts long-chain fatty acids into the pathways of oxidation and ketogenesis away from esterification, suggesting enhanced carnitine-dependent mitochondrial fatty acid uptake. The studies using octanoate indicated that norepinephrine also enhanced fatty acid oxidation by increasing the flux of acetyl-CoA through the Krebs cycle.

The data suggest that stress-associated sympathetic activation and norepinephrine discharge, as observed in diabetic ketoacidosis, result in direct activation of ketogenesis in the liver. DIABETES 1985; 34:774–79.

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Infusions of norepinephrine in man have been demonstrated to increase ketone body blood concentrations[1] and tracer-determined ketone body production.[2] The ketogenic effect of norepinephrine was larger than expected from the circulating free fatty acid concentrations, suggesting a direct stimulatory effect on hepatic ketogenesis.[2]

The role of catecholamines in the control of hepatic ketogenesis has not been clearly elucidated. Studies in rat hepatocytes demonstrated that norepinephrine enhanced fatty acid oxidation.[3] However, norepinephrine failed to influence ketogenesis in other reports.[4,5] Sugden et al. demonstrated even diminished ketone body production and enhanced fatty acid oxidation to CO_2 when hepatocytes were incubated with norepinephrine and mercaptopicolinate, an inhibitor of gluconeogenesis.[6]

Norepinephrine may enhance ketogenesis indirectly by its inhibitory influence on acetyl-CoA-carboxylase and subsequent inhibition of fatty acid synthesis.[7] Diminished fatty acid synthesis leads to decreased formation of malonyl-CoA, an important inhibitor of ketogenesis.[8]

The present studies were therefore performed to examine the capacity of norepinephrine to affect long-chain fatty acid oxidation to ketone bodies and to CO_2 and fatty acid esterification in isolated rat hepatocytes. To assess the effect of norepinephrine on carnitine-independent fatty acid metabolism, incubations were also performed using the medium-chain fatty acid, octanoate.[9] The receptor mediating norepinephrine's effect on fatty acid oxidation was determined by incubating norepinephrine combined with α- and β-receptor blockers.

MATERIALS AND METHODS

Hepatocyte isolation and incubation. Hepatocytes from male Wistar rats (300–350 g) having free access to chow (Nafag, Switzerland) were isolated at 11 a.m. by collagenase

TABLE 1
Effect of norepinephrine on oxidation and esterification of 0.5 mM [1-^{14}C]palmitate

	Controls (N = 10)	50 μM Norepinephrine (N = 10)
[1-^{14}C]palmitate conversion (nmol/mg/30 min) into		
CO_2	1.0 ± 0.2	2.1 ± 0.4*
Ketone bodies	1.5 ± 0.2	3.0 ± 0.4*
Triglycerides	12 ± 1	9.1 ± 0.8*
Mono-, diglycerides	2.1 ± 0.2	2.2 ± 0.3
Phospholipids	4.0 ± 0.3	4.0 ± 0.4
[1-^{14}C]palmitate uptake (nmol/mg/30 min)	25 ± 2	26 ± 2

*$P < 0.001$.

digestion of the perfused liver.[10] Liver cells (approximately 20 mg dry wt, equivalent to $\approx 10^7$ cells) were suspended in 2.3 ml Krebs-Ringer bicarbonate buffer containing 10 mM glucose and 1 g/dl defatted bovine serum albumin. The cell suspension was gassed with 95% O_2/5% CO_2 in 30-min intervals. Preliminary studies established that the effect of norepinephrine on hepatic palmitate oxidation and esterification was enhanced by preincubating liver cells with norepinephrine before the addition of palmitate. These studies demonstrated that preincubation of hepatocytes in Krebs-Ringer buffer per se for 60 min resulted in decreased [1-^{14}C]palmitate oxidation to CO_2 and acid-soluble products (mainly ^{14}C-ketone bodies) by 22% from 7.2 ± 0.9 to 5.6 ± 0.7 nmol [1-^{14}C]palmitate/mg liver cells · 30 min (mean ± SEM, N = 7). At the same time, [1-^{14}C]palmitate esterification to triglycerides increased by 11%. The preincubation period with norepinephrine with and without adrenergic blockers was 60 min, and the consecutive palmitate incubation period was 30 min unless stated otherwise. [1-^{14}C]labeled palmitate or octanoate was added as an albumin-bound complex at a final albumin concentration of 3.25 g/dl. [1-^{14}C]palmitate conversion into ketone bodies, acid soluble ^{14}C radioactivity, CO_2, and triglycerides was linear during 20–60 min of incubation. [1-^{14}C]octanoate conversion into ketone bodies and CO_2 was linear during 0–20 min of incubation. Cell viability as determined by trypan blue exclusion (trypan blue concentration: 4.5 mg/ml) was above 90% at the beginning of incubation, and decreased to about 80% after 180 min of incubation. Cellular [1-^{14}C]palmitate uptake and ketone body production from endogenous sources remained constant for 180 min after cell isolation.

Analyses. Extraction and separation of liver lipids was performed as described[9,11] using thin-layer chromatography in hexane : diethylether : acetic acid : methanol (90 : 21 : 2 : 3 by vol). The lipid fractions were scraped into scintillation vials.[12] Recoveries of labeled ^{14}C-triglycerides, ^{14}C-phospholipids, and ^{14}C-palmitate added to liver cell suspensions were over 80%. For determination of $^{14}CO_2$, 0.3 ml perchloric acid 30% was injected through the rubber stopper of the Erlenmeyer flasks. The flasks were bubbled with CO_2-free air for 30 min during constant shaking and outflowing air was conducted into a CO_2-absorbent scintillator (CO_2-Oxyfluor). Contamination of $^{14}CO_2$ counts by ^{14}C-acetone was <2%. The perchloric acid supernatant was neutralized with KOH and used for determination of acid-soluble ^{14}C products and ^{14}C-total ketone bodies[13] with a minor modification.[14] Since under the present experimental conditions equal labeling of the carboxyl and carbonyl groups of acetoacetate can be expected,[15] only ^{14}C-acetone was measured, and its radioactivity was multiplied by two for calculation of total ^{14}C-ketone body radioactivity. Mean recovery of added $^{14}CO_2$, ^{14}C-β-hydroxybutyrate, and ^{14}C-acetoacetate were 95%, 80%, and 90%, respectively. Unlabeled β-hydroxybutyrate and acetoacetate were determined enzymatically.[16] Norepinephrine concentrations of incubation media were determined by radioenzymatic assay.[17]

Materials. Collagenase type IV, yohimbine HCl, and DL-propranolol were purchased from Sigma, St. Louis, Missouri. All radioactive compounds and scintillation fluids were from New England Nuclear, Boston, Massachusetts. Defatted bovine serum albumin and L-norepinehrine HCl were from Fluka, Switzerland. Octanoate, palmitate, and TLC plates (silica gel 60, 0.25 mm with concentrating zone) were from Merck, FRG. Prazosine was a gift from Pfizer, Switzerland. Palmitate-albumin complexes were prepared according to Spector and Hoak.[18]

Presentations of data. [1-^{14}C]fatty acid conversion (nmol/mg liver cells [dry wt]/min) into CO_2, and total ketone bodies and lipids were calculated by dividing the radioactivity of these products by the [1-^{14}C]fatty acid specific activity. Uptake of added [1-^{14}C]palmitate was calculated from the difference of [1-^{14}C]palmitate contained in the medium before and after incubation with liver cells. The data were evaluated statistically by Student's paired *t*-test. Results represent means ± SEM. Data of N observations refer to N different rats.

RESULTS

Effect of norepinephrine on oxidation and esterification of 0.5 mM [1-^{14}C]palmitate (Table 1). Norepinephrine enhanced the conversion of [1-^{14}C]palmitate into CO_2 and

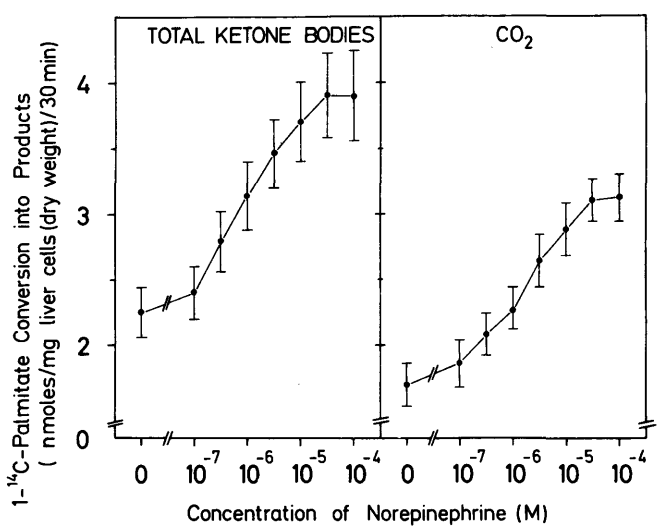

FIGURE 1. Dose dependence of norepinephrine's effect on [1-^{14}C]-palmitate conversion into total ketone bodies and CO_2. Preincubation with norepinephrine and incubation with [1-^{14}C]palmitate were as described in the text. Results are means ± SEM of 5–7 experiments.

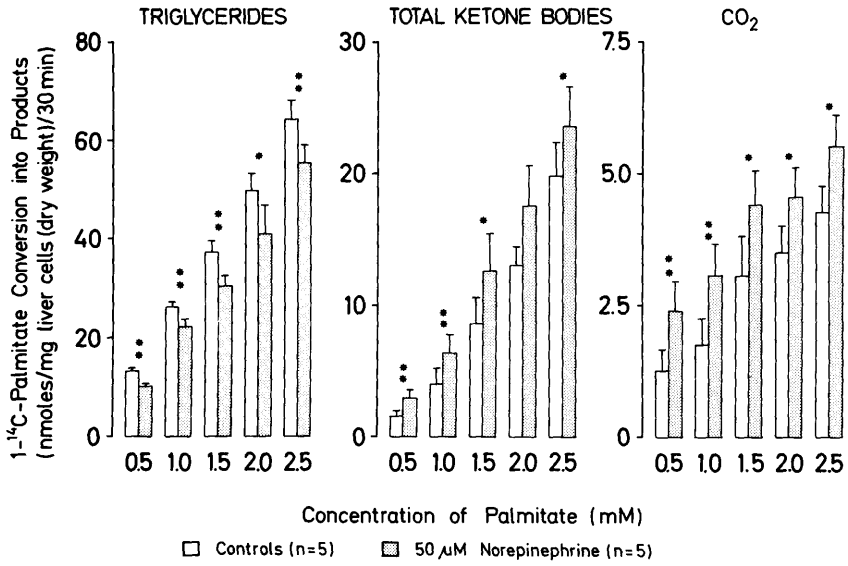

FIGURE 2. Effect of norepinephrine (50 μM) on conversion of [1-^{14}C]palmitate into ketone bodies, CO_2, and triglycerides at increasing concentrations of palmitate. Incubation conditions are described in the text. * $P < 0.05$, ** $P < 0.01$.

ketone bodies, and decreased palmitate esterification to triglycerides. The norepinephrine-induced decrease in palmitate esterification was quantitatively sufficient to explain the observed increase in palmitate conversion into CO_2 and ketone bodies. Norepinephrine failed to affect [1-^{14}C]-palmitate incorporation into mono- and diglycerides, and into phospholipids. [1-^{14}C]palmitate uptake by liver cells was not influenced by norepinephrine.

Concentration dependence of norepinephrine's effect on [1-^{14}C]palmitate conversion into CO_2 and ketone bodies, and degradation of norepinephrine by hepatocytes (Figure 1). Figure 1 demonstrates that norepinephrine concentrations between 10^{-7} and 10^{-4} M resulted in progressive enhancement of conversion of 0.5 mM [1-^{14}C]palmitate into ketone bodies and CO_2. The minimal effective dose was 10^{-7} M, and the maximal effect was observed at 5×10^{-5} M norepinephrine. These data were obtained at a liver cell density of approximately 7 mg dry wt/ml. The dose-response curve was influenced by the cell density during incubation; when incubations were performed at higher cell density (28 mg dry wt/ml, N = 5), the half-maximal effect of norepinephrine on [1-^{14}C]palmitate conversion to CO_2 exceeded 10^{-4} M norepinephrine. Measurement of norepinephrine concentrations before and after incubation using radioenzymatic assay demonstrated considerable degradation of norepinephrine during incubation. Incubation of 60 min with norepinephrine (50 μM) at a liver cell density of 5.5 ± 0.1 mg/dry wt/ml resulted in 92 ± 1% degradation of the added norepinephrine (N = 3); during incubation with a cell density of 28 mg/dry wt/ml, 99.9 ± 0.02% of the added norepinephrine was degraded during 60 min of incubation. Control incubations with buffer and without hepatocytes demonstrated that only 17 ± 7% of added norepinephrine was degraded during 60 min.

Influence of norepinephrine on [1-^{14}C]palmitate oxidation and esterification at increasing palmitate concentrations (Figure 2). Figure 2 demonstrates that increasing palmitate concentrations from 0.5 to 2.5 mM resulted in augmented [1-^{14}C]fatty acid conversion into ketone bodies, CO_2, and triglycerides, both in controls and in norepinephrine-treated cells. Norepinephrine increased [1-^{14}C]palmitate conversion into ketone bodies and CO_2, and decreased esterification significantly at all palmitate concentrations studied. The relative increase in [1-^{14}C]palmitate conversion into ketone bodies and CO_2 by norepinephrine was greatest (93%) at 0.5 M palmitate.

[1-^{14}C]palmitate uptake increased linearly from 29 ± 3 to 140 ± 11 nmol/mg/30 min when the palmitate concentration was raised from 0.5 to 2.5 mM. Palmitate uptake was uninfluenced by norepinephrine. Increasing palmitate concentration in the incubation medium from 0.5 to 2.5 mM in control studies resulted in a relative augmentation of ketogenesis and diminution of palmitate esterification, since conversion of [1-^{14}C]palmitate into ketone bodies increased from

TABLE 2
Effect of norepinephrine on production of labeled and unlabeled ketone bodies

	Controls	Norepinephrine (50 μM)
	(nmol/mg/30 min, N = 10)	
Production of unlabeled ketone bodies (0.5 mM palmitate added)	32 ± 4	39 ± 6†
[1-^{14}C]palmitate converted into ketone bodies	1.5 ± 0.2	3.0 ± 0.4§
Percent of ketone bodies derived from added palmitate*	20 ± 2	35 ± 5‡
Endogenous ketone body production (production of unlabeled ketone bodies without added palmitate)	23 ± 3	22 ± 3

*Calculated from: % = (nmol [1-^{14}C]palmitate converted into ketone bodies [×4])/(nmol unlabeled ketone bodies produced) × 100.
†$P < 0.05$, ‡$P < 0.01$, and §$P < 0.001$.

TABLE 3
Effect of norepinephrine (NE) on conversion of [1-^{14}C]octanoate into CO_2 and ketone bodies

	Controls (N = 5)	50 μM NE (N = 5)
0.1 mM [1-^{14}C]octanoate conversion into:		
CO_2	1.0 ± 0.1	1.5 ± 0.1*
Ketone bodies	2.2 ± 0.2	2.0 ± 0.1
0.5 mM [1-^{14}C]octanoate conversion into:		
CO_2	1.9 ± 0.1	2.9 ± 0.2†
Ketone bodies	22 ± 1	24 ± 1

Expressed as nmol octanoate converted per mg liver cells, dry wt, per 10 min.
*$P < 0.01$, †$P < 0.001$ versus controls.

6.1 ± 1.0 to 14 ± 2% of [1-^{14}C]palmitate uptake, whereas [1-^{14}C]palmitate conversion into esterified fatty acids decreased from 72 ± 5% to 56 ± 6% of [1-^{14}C]palmitate uptake.

Effect of norepinephrine on production of unlabeled ketone bodies (Table 2). Norepinephrine exerted a significant stimulatory effect on production of unlabeled ketone bodies. However, the relative increase of ketogenesis was smaller (23%) compared with that of the conversion of 0.5 mM [1-^{14}C]palmitate into ketone bodies (99%). Only 20 ± 2% of total ketone body production resulted from conversion of added palmitate into ketone bodies in control studies, the remainder originating from other sources, e.g., endogenous lipids. The results indicated no significant effect of norepinephrine on ketogenesis from these endogenous precursors.

Effect of norepinephrine on conversion of [1-^{14}C]-octanoate into ketone bodies and CO_2 (Table 3). Incubation with norepinephrine increased conversion of 0.1 and 0.5 mM [1-^{14}C]octanoate into CO_2 rapidly and significantly, whereas ketogenesis was not affected.

Effect of α- and β-receptor blocking agents on norepinephrine's effect on fatty acid oxidation (Table 4). The norepinephrine effect on [1-^{14}C]palmitate oxidation and ketogenesis was inhibited by the α_1-blocker prazosine but not by the α_2-blocker yohimbine or by the β-blocker propranolol, suggesting that the norepinephrine effect on palmitate oxidation was α_1-receptor mediated. In control experiments without norepinephrine, these blockers failed to influence [1-^{14}C]palmitate oxidation.

The effect of norepinephrine on conversion of [1-^{14}C]octanoate (0.5 mM) into CO_2 was also inhibited by prazosine; [1-^{14}C]octanoate conversion to CO_2 was 2.2 ± 0.1 in controls, and 2.2 ± 0.2 nmol/mg/10 min during 1 μM norepinephrine + 10^{-8} M prazosine, respectively, whereas norepinephrine alone increased [1-^{14}C]octanoate oxidation to CO_2 to 3.2 ± 0.3 nmol/mg/10 min ($P < 0.01$). Yohimbine or propranolol failed to inhibit norepinephrine's effects on [1-^{14}C]octanoate oxidation to CO_2.

DISCUSSION

The present results demonstrate the ability of norepinephrine to divert long-chain fatty acids into the pathways of ketogenesis and CO_2 production, away from esterification to triglycerides. This finding, and the observation that norepinephrine failed to increase ketogenesis from medium-chain fatty acids, suggests that norepinephrine enhances carnitine acyltransferase-mediated mitochondrial uptake of long-chain fatty acids.[9,19]

A further effect of norepinephrine was an increase in [1-^{14}C]octanoate oxidation to CO_2.

The observation that norepinephrine diminished palmitate esterification to triglycerides, but not to other fatty acid esters, suggests that norepinephrine also exerted a regulatory effect on the branching point between triglyceride and phospholipid synthesis.[20]

Previous studies on norepinephrine's effect on hepatic fatty acid metabolism reported controversial results. While norepinephrine exerted a modest stimulatory effect on conversion of ^{14}C-fatty acids into acid-soluble products in isolated rat hepatocytes,[3] no effect of the catecholamine on ketone body production was observed in a similar experimental model.[4,5] This discrepancy may be due to several reasons: first, in the latter reports the experiments were carried out at higher fatty acid concentrations, which, per se, are ketogenic[21] and decrease norepinephrine's effect on ketogenesis (Figure 2). Second, Sugden et al.[4] used juvenile rats in their experiments, which have been demonstrated to differ from mature rats as used in the present studies regarding responsiveness to α- and β-adrenergic stimuli.[22] Third, since net total ketone body production was less enhanced by norepinephrine than was ketone body production from ^{14}C-labeled fatty acids (Table 2), its use as parameter of ketogenesis[4] may have rendered the detection of norepinephrine's ketogenic effect difficult. This may have been due to the fact that unlabeled ketone bodies were also produced from precursors other than long-chain fatty acids. Since ketogenesis from these precursors is carnitine-independent, their oxidation was not enhanced by norepinephrine. This observation further supports the assumption that norepi-

TABLE 4
Effect of α- and β-receptor blockers on norepinephrine's effect on palmitate oxidation and on ketogenesis

	Conversion of [1-^{14}C]palmitate (nmol/mg/30 min) into:	
	CO_2	Total ketone bodies
Controls	1.4 ± 0.2	1.8 ± 0.3
3×10^{-6} M NE	2.3 ± 0.3	2.8 ± 0.5
3×10^{-6} M NE + 10^{-7} M prazosine	1.4 ± 0.2	1.7 ± 0.3
+ 10^{-8} M prazosine	1.8 ± 0.3	2.1 ± 0.4
+ 10^{-9} M prazosine	2.0 ± 0.2	2.6 ± 0.6
+ 10^{-7} M yohimbine	2.3 ± 0.3	2.7 ± 0.5
+ 3×10^{-5} M propranolol	2.4 ± 0.4	2.8 ± 0.5

nephrine influenced carnitine-dependent fatty acid metabolism. Fourth, norepinephrine was rapidly degraded by hepatocytes in the present experiments, probably due to the high content of catecholamine o-methyltransferase present in liver.[23] Catecholamine degradation was augmented when incubation was performed with high liver cell density as used in a previous study.[5] Taking degradation during incubation into account, the present effects of norepinephrine were observed at similar concentrations (10^{-7} M), as occurs in plasma during pathophysiologic conditions.[24]

Fifth, Burrin et al.[5] used carnitine supplementation, yielding supraphysiologic concentrations in their hepatocyte incubations.[5] This may have increased conversion of fatty acids into ketone bodies,[25] thereby abolishing stimulatory hormonal effects.[26]

The point may be raised that carnitine concentrations were presumably decreased during incubation of isolated hepatocytes[27] and thereby influenced the present results. In spite of this observation, carnitine supplementation was not used in the present study, in agreement with previous reports[26,28] demonstrating stimulatory effects of glucagon on carnitine-dependent fatty acid oxidation. During the 60 min of preincubation, carnitine depletion was unlikely to have occurred, since fatty acid oxidation decreased in control studies by only 22%. Furthermore, the notion that fatty acid oxidation rates were of similar magnitude as those observed by others[8,26,28] suggests that carnitine depletion was insignificant during the present incubation conditions.

The relative increase in ketogenesis from [1-^{14}C]palmitate by norepinephrine in the present study was largest at low (0.5 mM) palmitate concentrations, possibly due to the fact that elevated palmitate concentrations increased [1-^{14}C]palmitate conversion into ketone bodies. This observation is in agreement with previous reports,[21] and is probably related to a fall in malonyl-CoA content.[28] It appears, therefore, that norepinephrine plays a role in activating ketogenesis early in the transition from a nonketotic to a ketotic state.

Alpha-adrenergic agonists enhanced [1-^{14}C]octanoate oxidation to CO_2,[29] in agreement with the present data. In contrast, our results indicated that ketogenesis from octanoate was unaffected, suggesting that norepinephrine increased fatty acid oxidation by a second effect independent of its influence on the intrahepatic partitioning of long-chain fatty acids between ketogenesis and esterification. Stimulation of [1-^{14}C]octanoate oxidation to CO_2 by norepinephrine suggests augmented fatty acid oxidation subsequent to β-oxidation, e.g., increased Krebs cycle flux. This effect has been suggested to be related to a catecholamine-induced increase in oxaloacetate availability in mitochondria, thereby augmenting acetyl-CoA oxidation to CO_2.[6]

An alternative explanation for the norepinephrine effect on [1-^{14}C]octanoate oxidation to CO_2 may be related to its inhibitory effect on lipogenesis.[7] Since oxidation to acetyl-CoA and lipogenesis is a major pathway of octanoate metabolism,[9] inhibition of lipogenesis by norepinephrine may increase the availability of acetyl-CoA for oxidation to CO_2.

The present results demonstrate that norepinephrine's effect on ketogenesis from long-chain fatty acids and on octanoate oxidation to CO_2 was α_1-receptor mediated, implying Ca^{2+}-dependence of the intracellular effect.[30]

Thus, α_1-receptor stimulation influenced the partitioning of fatty acids into the pathways of oxidation away from esterification, in a manner similar to cAMP-mediated effectors such as glucagon.[31,32] The common mechanism of cAMP and Ca^{2+}-dependent stimulation of ketogenesis may be an inactivation of acetyl-CoA carboxylase, which has been reported to be influenced by cAMP[33] and by Ca^{2+}.[7] The resulting decreased malonyl-CoA formation would increase fatty acid oxidation.[8] Alternatively, malonyl-CoA synthesis may be diminished due to inhibition of glycolysis, e.g., by decreased fructose-2,6-bisphosphate.[34] Further studies are currently in progress to further clarify norepinephrine's mechanisms of action. The present results demonstrate that norepinephrine regulates hepatic fatty acid metabolism at three sites, namely by augmenting fatty acid oxidation and ketogenesis, by diminishing esterification to triglycerides, and by enhancing oxidation of fatty acid-derived acetyl-CoA to CO_2. The present data correspond to previous findings in humans[2] that norepinephrine may increase hepatic ketone body production directly. This suggests that sympathetic activation during stress (e.g., diabetic ketoacidosis) results in norepinephrine-induced acceleration of hepatic ketogenesis.

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