The Regulation of Plasma Ketone Body Concentration by Counter-Regulatory Hormones in Man

III. Effects of Norepinephrine in Normal Man

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SUMMARY

We examined the mechanism of norepinephrine's ketogenic activity in normal man. The contribution of norepinephrine's lipolytic activity to the subsequent ketosis was assessed by comparing the degree of ketosis attained during hormone infusion and the degree of ketosis observed after heparin-induced free fatty acid substrate generation. In addition, we examined the effect of norepinephrine infusion on the plasma concentration of other stress hormones (glucagon, cortisol, and growth hormone) with the experimental protocol.

The results obtained during norepinephrine infusion into normal man demonstrated that norepinephrine's lipolytic activity was not the principal determinant of its ketogenic activity. In fact, less than 50% of norepinephrine's ketogenic activity could be attributed to the rise in plasma free fatty acid concentration. Furthermore, the ketosis that resulted from plasma norepinephrine elevation may have been a direct hormonal effect at the liver or may have been secondary to the norepinephrine-induced elevation of glucagon and/or suppression of insulin.

Our results emphasize the important role that endogenous insulin secretion plays on modulating the ketogenic effects of the stress hormones. Thus, norepinephrine, which suppresses plasma insulin secretion, is markedly ketogenic compared with the normal ketogenic response to glucagon, in spite of the fact that both of these stress hormones may activate ketogenesis via cyclic AMP formation. In conclusion, this study, in concert with a previous study in diabetic man, firmly established norepinephrine as a major ketogenic hormone in man. DIABETES 28:5–10, January 1979.

Norepinephrine is a counter-regulatory hormone in man that has been implicated in the pathogenesis of diabetic ketoacidosis.[1,2] Infusion of norepinephrine into normal man at physiologic concentrations has resulted in both lipolysis and ketosis.[3] However, whether this elevation of plasma ketone body concentration in normal man is a primary norepinephrine-induced effect or is only secondary to the elevation of plasma free fatty acid substrate is not known.[4] Furthermore, whether this ketogenic action may be modulated by other norepinephrine-induced counter-regulatory hormones and/or insulin secretion is not resolved.[3,5]

In order to explore the ketogenic effect of norepinephrine, we infused norepinephrine into nondiabetic man in high physiologic concentrations to induce ketosis and we monitored simultaneously the changes in plasma insulin and glucagon. The degree of ketosis observed was compared with the degree of ketosis obtained when free fatty acids were elevated by heparin administration to a similar degree as observed during norepinephrine infusion. This control-heparin study provided a means of assessing the ketogenic effect of norepinephrine in man independent of its effect in elevating free fatty acid substrate concentration.

METHODS

Subject population. Six healthy subjects (four men and two women) participated in both the control-heparin and norepinephrine infusion studies. Ages of the volunteers ranged from 21 to 34 years, and all subjects were within 5% of their ideal body weight.[6] No subject was receiving medication nor was diabetic during a 100-g oral glucose tolerance test by criteria of U.S. Public Health Service.[7] Informed consent was obtained by all participants, and no adverse side effects occurred during these studies. The order in which the studies were completed was randomized, but for any one individual, at least a one-week interval occurred between the control-heparin study and the norepinephrine infusion study.

Study protocol. All studies were completed between 0600 and 0800 h after an overnight 12-h fast and before breakfast. After assuming the supine position, a no. 19 scalp

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vein needle was inserted into a large antecubital vein of each arm. Patency of these veins was maintained by the continuous infusion of normal saline at a rate of 1 ml/min via a Harvard infusion pump. After a 30-min baseline period, either heparin (in the control-heparin study) or norepinephrine (in the norepinephrine infusion study) was administered into one of these antecubital veins. The heparin was administered as a 5000-U bolus, whereas the norepinephrine was infused at a concentration of 0.04 μg/kg/min at a rate of 1 ml/min in a solution of normal saline containing 1% human albumin. This concentration of norepinephrine was calculated to attain a plasma concentration of about 1000 pg/ml,[8] a plasma concentration frequently obtained during stress.[1,8–10]

All blood samples were obtained retrograde from the contralateral scalp vein needle after discarding the initial 2 ml of blood. Throughout the 30-min baseline period and the following 60-min experimental period, a total of 14 10-ml blood samples was obtained at the times indicated in Table 1. Thus, throughout the entire 90-min study period, a total of 140 ml of blood was removed and a total of 180 ml of normal saline was infused.

Assay of substrates and hormones. Immediately after withdrawal of the 10-ml blood sample, the blood was placed in a 10-ml heparinized Vacutainer test tube containing 100 μl of 1.0 M benzamidine as a preservative.[11] This blood was rapidly chilled to 4°C in an ice bath. Previous studies have demonstrated that this procedure effectively prevented the hydrolysis in vitro of endogenous triglycerides by heparin-activated lipoprotein lipase.[5] The blood was then separated by centrifugation and the plasma was removed for multiple substrate and hormonal assay.

Plasma ketone bodies were assayed on the day of study by standard enzymatic analysis.[12] The plasma for all other

TABLE 1
Concentrations of hormones and substrates during control saline infusion and norepinephrine infusion

Hormones/ substrates	Baseline period −30 (min)	−25	−20	−10	Basal sample −1	Heparin injection ↓	Experimental period +2 (min)	+5	+10	+15	+20	+30	+40	+50	+60
I. Control saline infusion															
Acetoacetate (μmol/L)	115 ± 32	118 ± 39	139 ± 52	133 ± 35	115 ± 30		120 ± 34	130 ± 38	141 ± 43	121 ± 36	144 ± 34	152 ± 40	157 ± 43	153 ± 29	149 ± 31
Betahydroxybutyrate (μmol/L)	133 ± 51	126 ± 33	169 ± 67	147 ± 36	176 ± 77		176 ± 76	180 ± 82	172 ± 64	219 ± 68	223 ± 39	220 ± 43	220 ± 46	215 ± 54	213 ± 45
Glucose (mg/dl)	86 ± 2	84 ± 1	84 ± 1	84 ± 1	83 ± 1		84 ± 2	81 ± 1	80 ± 2	80 ± 1	82 ± 1	84 ± 1	83 ± 2	87 ± 2	86 ± 2
Cortisol (μg/dl)	18 ± 5	17 ± 4	16 ± 3	17 ± 4	14 ± 3		16 ± 4	15 ± 3	14 ± 3	17 ± 5	16 ± 5	16 ± 5	15 ± 5	14 ± 5	13 ± 5
Growth hormone (ng/ml)	4 ± 1	3 ± 1	3 ± 1	3 ± 1	3 ± 1		3 ± 1	2 ± 0.4	2 ± 0.3	2 ± 0.3	2 ± 0.3	2 ± 0.3	2 ± 0.3	2 ± 0.3	2 ± 0.3
Epinephrine (pg/ml)				42 ± 16	54 ± 14				44 ± 14			44 ± 14			38 ± 18
Norepinephrine (pg/ml)				300 ± 40	312 ± 39				365 ± 92			332 ± 71			354 ± 90
						Norepinephrine infusion ↓									
II. Norepinephrine infusion															
Acetoacetate (μmol/L)	58 ± 13	72 ± 16	71 ± 15	86 ± 22	92 ± 25		55* ± 13	59 ± 17	78 ± 26	96 ± 37	124 ± 42	162 ± 33	211 ± 60	215 ± 60	195 ± 45
Betahydroxybutyrate (μmol/L)	96 ± 11	135 ± 18	152 ± 20	191* ± 38	163 ± 41		140 ± 29	144 ± 18	165 ± 32	265 ± 57	358* ± 86	521* ± 91	643* ± 133	634* ± 117	598* ± 113
Glucose (mg/dl)	83 ± 2	86 ± 2	87 ± 2	88* ± 2	88* ± 2		86 ± 3	92* ± 3	96* ± 2	99* ± 3	99* ± 2	100* ± 3	100* ± 4	99* ± 4	101* ± 4
Cortisol (μg/dl)	18 ± 3	16 ± 4	17 ± 3	16 ± 4	14 ± 4		14 ± 4	16 ± 3	14 ± 3	13 ± 3	14 ± 5	11 ± 3	11 ± 3	10 ± 3	12 ± 3
Growth hormone (ng/ml)	2* ± 0.2	2* ± 0.2	2 ± 0.4	2 ± 0.4	2 ± 0.4		2 ± 0.4	2 ± 0.5	2 ± 0.5	2 ± 0.6	2 ± 0.6	2 ± 0.5	2 ± 0.5	2 ± 0.4	2 ± 0.4
Epinephrine (pg/ml)				42 ± 12	39 ± 15				39 ± 15			39 ± 15			39 ± 15
Norepinephrine (pg/ml)				321 ± 45	311 ± 22				1268* ± 136			1500* ± 116			1224* ± 135

* $P < 0.05$ compared with the control study.

assays was aliquoted into individual test tubes and frozen to −20°C until the time of assay. For each individual subject, the frozen plasma samples from both the control-heparin and the norepinephrine infusion study were included in the same assay in order to reduce interassay variation. Plasma glucose concentration was assayed by glucose oxidase using a Beckman glucose analyzer.[13] Plasma free fatty acids were measured by the method of Dole[14] as modified by Duncombe.[15] Insulin was measured by two-antibody radioimmunoassay[16] and glucagon by the method of Unger.[17] Cortisol and growth hormone were assayed by radioimmunoassay by the methods of Foster and Dunn[18] and Peake,[19] respectively. Blood for both epinephrine and norepinephrine assays was collected in special Vacutainer test tubes containing heparin as the anticoagulant and reduced glutathione as the reducing agent. These specimens were immediately centrifuged and the plasma was stored at −20°C until assayed. The assay for catecholamines was performed according to the method of Peuler and Johnson[20] with the thin-layer chromatography solvent modifications of Da Prada and Zurcher.[21]

Since each subject participated in both a control-heparin and a norepinephrine infusion study, a paired Student's *t* test was used to compare the corresponding concentrations in the two studies. Integration of the areas under the curves was performed with a Hewlett-Packard 9815 desk-top computer.

RESULTS

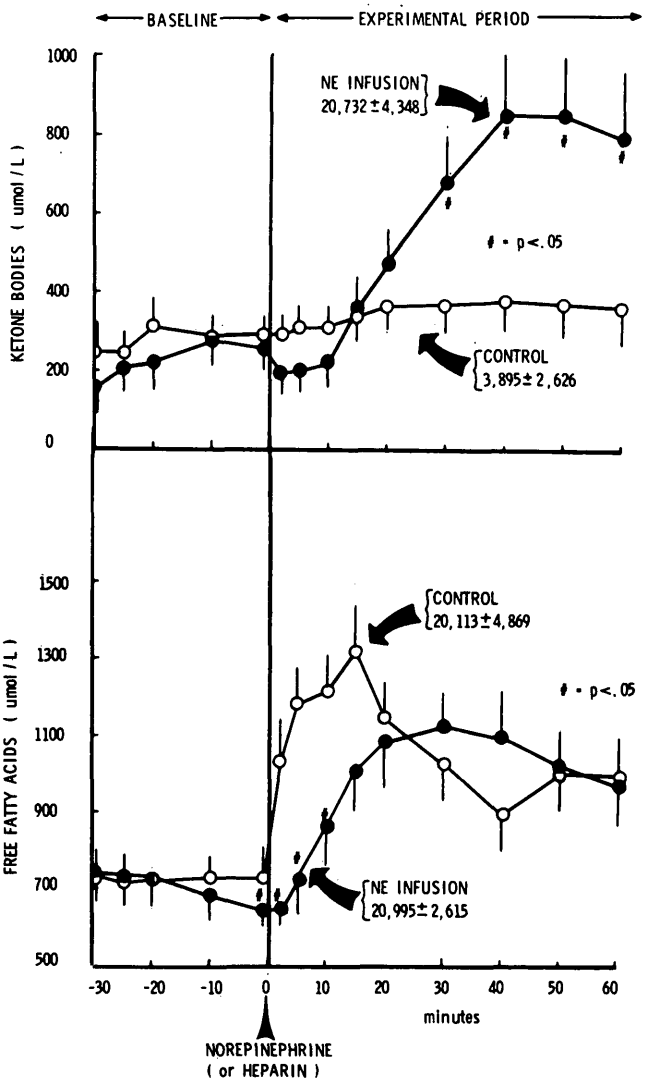
The entire study required 90 min to complete. The first 30 min was termed the baseline period, and the following 60 min was termed the experimental period. The sample obtained immediately before the experimental period (at −1 min) was designated the basal sample and was the concentration by which the baseline periods in the control-heparin and norepinephrine infusion studies were compared. The data are expressed as the means ± SE of the six subjects in each study.

SUBSTRATES

Free fatty acids (Figure 1). In the control-heparin study, the basal plasma free fatty acid concentration was 723 ± 20 μmol/L, which was not statistically different from the basal plasma free fatty acid concentration in the norepinephrine infusion study (662 ± 46 μmol/L) ($P > 0.05$). As shown in Figure 1, immediately after the bolus heparin injection in the control-heparin study, a rapid increase in plasma free fatty acids was observed that was statistically greater than the plasma free fatty acid concentration in the norepinephrine infusion study at three corresponding observation points (2, 5, and 10 min) ($P < 0.05$). After the initiation of norepinephrine infusion, a gradual increase in plasma free fatty acids was observed that obtained its maximum concentration at +30 minutes. At no observation point did the mean plasma concentration of free fatty acids during norepinephrine infusion study exceed the corresponding concentration in the control-heparin study ($P > 0.05$). As depicted in Figure 1, when the integrated areas above basal concentration were calculated for the entire 60-min experimental period, the integrated rise in plasma free fatty acid concentration for the control-heparin study (20,113 ± 4869 μmol/L-min) was not different from the integrated rise during the norepinephrine infusion study (20,995 ± 2615 μmol/L-min) ($P > 0.1$). Thus, from these calculations, the availability of plasma free fatty acid substrate to support hepatic ketogenesis was not different in the two studies during the entire 60-min experimental period.

Ketone body concentration (Figure 1, Table 1). As depicted in the upper half of Figure 1, basal plasma total ketone body concentration (acetoacetate plus betahydroxybutyrate) was not statistically different in the control-heparin study (290 ± 98 μmol/L) compared with the basal concentration in the norepinephrine infusion study (255 ± 68 μmol/L) ($P > 0.1$). During the experimental period, a gradual increase in plasma ketone body concentration was observed in both the control-heparin and the norepinephrine infusion studies. Although the mean plasma ketone body concentration attained a maximum concentration at +40 minutes in both studies, the plasma ketone body concentration observed in the norepinephrine infusion study significantly ex-

FIGURE 1. The effect of norepinephrine infusion on plasma ketone body (top) and free fatty acid concentration (bottom). Integrated plasma free fatty acid concentration was not statistically different ($P < 0.05$) in the norepinephrine infusion study (solid circles) compared with the control-heparin study (open circles). In contrast, the plasma concentration of ketone bodies in the norepinephrine infusion study greatly exceeded the plasma concentration of ketone bodies in the control-heparin study (top).



ceeded the concentration observed in the control-heparin study at four observation points (+30, +40, +50, and +60 min) by a minimum of 400 μmol/L ($P < 0.01$).

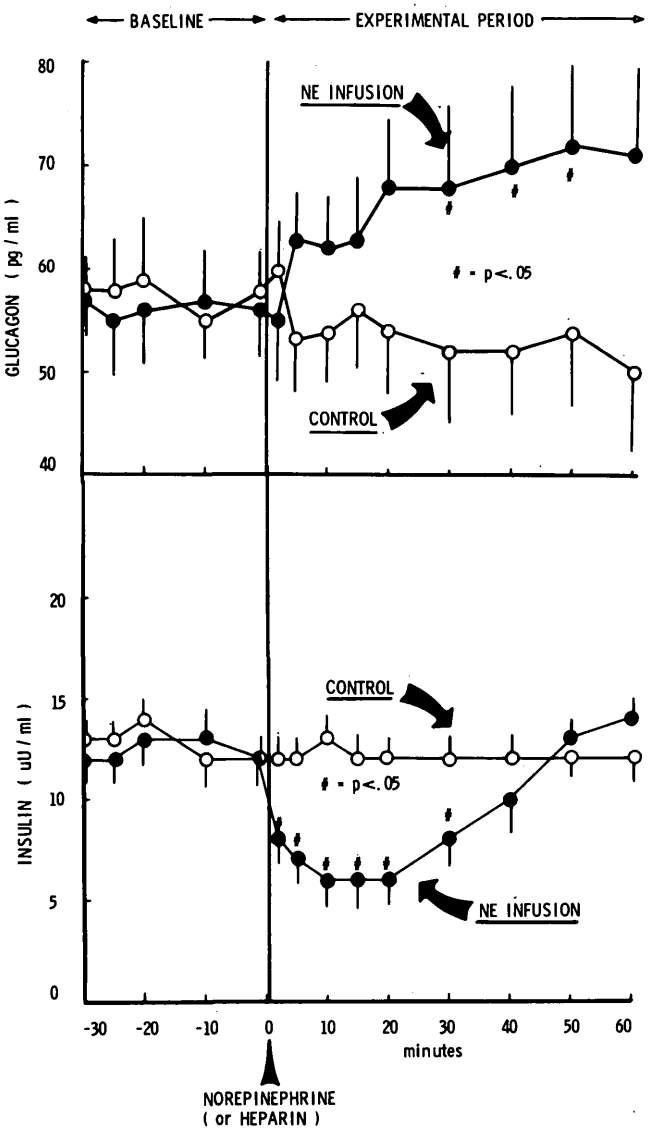
When the integrated rise above basal concentration was calculated for the entire 60-min experimental period, the elevation of plasma ketone body concentration in the norepinephrine infusion study (20,732 ± 4,348 μmol/L-min) was approximately fivefold greater than that observed in the control-heparin study (3895 ± 2626 μmol/L-min) ($P < 0.01$). Thus, as is evident from Figure 1, norepinephrine infusion resulted in a significantly greater ketosis than can be accounted for solely by the norepinephrine-induced elevation of free fatty acid substrate concentration.

The changes in the plasma concentration of the individual ketone bodies (acetoacetate and betahydroxybutyrate) are given in Table 1. In general, these individual ketone bodies followed the same pattern of change as the total ketone bodies.

Plasma glucose concentration (Table 1). Basal plasma glucose concentration in the control-heparin study was 83 ± 1 mg/dl compared with a corresponding concentration of 88 ± 2 mg/dl in the norepinephrine infusion study. Although this difference obtained statistical significance ($P < 0.05$) at three other observation times during the baseline period (−30, −25, and −20 min), the plasma glucose concentrations in the two studies were not different ($P > 0.05$).

During the 60-min experimental period, no statistically significant change in mean plasma glucose was observed in the control-heparin study (Table 1). This lack of change contrasted to the progressive increase in plasma glucose concentration that was observed during the norepinephrine infusion study (Table 1). This progressive increase in plasma glucose concentration was statistically greater than the corresponding concentration in the control-heparin study at eight of the nine observation times. Thus, in all our study subjects, norepinephrine infusion resulted in a rise of plasma glucose to levels exceeding 100 mg/dl.

FIGURE 2. The effect of norepinephrine infusion on plasma glucagon (top) and insulin (bottom) concentration. Norepinephrine infusion (solid circles) resulted in an elevation of plasma glucagon concentration and a suppression of plasma insulin concentration compared with the control-heparin study (open circles) ($P < 0.05$).



HORMONES

Plasma insulin concentration (Figure 2). As given in Figure 2, basal plasma insulin concentration in the control-heparin study (12 ± 1 μU/ml) was not different from that of the norepinephrine infusion study (12 ± 1 μU/ml) ($P < 0.1$). As depicted in the upper half of Figure 2, during the 60-min experimental period in the control-heparin study, only minor variations in plasma insulin concentration were observed. This stable level of circulating insulin contrasted to that observed in the norepinephrine infusion study. In this latter study, a definitive decline in plasma insulin concentrations was observed that attained significance at six observation points. After this initial suppression of plasma insulin concentration, a gradual return toward basal concentration was observed during the latter 30 min of the experimental period.

Plasma glucagon concentration (Figure 2). Basal plasma glucagon concentration was within the normal range for our laboratory (50 to 100 pg/ml) in both the control-heparin (58 ± 14 pg/ml) and the norepinephrine infusion study (56 ± 6 pg/ml) ($P > 0.05$). As shown in the bottom half of Figure 2, during the experimental period no significant change in plasma glucagon concentration was observed. This lack of change contrasted to that observed during the norepinephrine infusion study, in which a progressive increase in plasma glucagon concentration occurred. This elevation of plasma glucagon to 70 ± 9 and 71 ± 10 pg/ml was statistically greater than the corresponding concentration in the control-heparin study of 52 ± 8 and 54 ± 9 pg/ml at two observation points (+40 and +50 min), $P < 0.05$.

Plasma cortisol concentration (Table 1). As given in Table 1, basal plasma cortisol concentration was not different in the control-heparin study (14 ± 3 μg/dl) compared with the basal concentration in the norepinephrine infusion study (14 ± 4 μg/dl) ($P > 0.1$). Although minor variations in plasma cortisol concentration were observed throughout the experimental period in both the control-heparin and norepinephrine infusion studies, at no observation point was the concentration statistically different in the two studies ($P > 0.05$).

Plasma growth hormone concentration (Table 1). As given in Table 1, mean basal plasma growth hormone concentration was within our normal range (<10 μg/ml) in both

the control-heparin and norepinephrine infusion studies. Although at the start of the baseline period (−30 and −25 min) the plasma growth hormone concentration in the control-heparin study exceeded that in the norepinephrine infusion study (Table 1), the basal concentration in the control-heparin study (3 ± 1 ng/ml) was not different from the basal concentration in the norepinephrine infusion study (2 ± 0.4 ng/ml) ($P < 0.05$). During the 60-min experimental period in both studies, no significant changes in growth hormone concentration were observed ($P < 0.05$).

Plasma catecholamine concentration (Table 1). As depicted in Table 1, basal plasma epinephrine concentration in the control-saline infusion study (54 ± 14 pg/ml) was not significantly different statistically from that of the norepinephrine infusion study (39 ± 15 pg/ml) ($P > 0.05$). Furthermore, no significant change in mean plasma concentration occurred during the infusion of norepinephrine. Table 1 also demonstrates that no significant difference in basal plasma norepinephrine concentration was observed in the control-saline study (312 ± 39 pg/ml) versus the norepinephrine infusion study (311 ± 22 pg/ml) ($P > 0.05$). However, during the infusion of norepinephrine into the volunteers, plasma norepinephrine concentration rose and obtained concentrations between 1200 and 1500 pg/ml at the three observation points (10, 30, and 60 min) ($P < 0.05$ compared with corresponding control concentrations).

DISCUSSION

This study demonstrates that norepinephrine infusion into nondiabetic man results in an elevation of plasma ketone body concentration that may be attributed in part to a lipolytic effect and in part to a separate ketogenic effect. This ketogenic effect of norepinephrine may be a direct effect of the hormone or may be mediated, at least partially, by its simultaneous suppression of plasma insulin concentration and/or elevation of plasma glucagon concentration.

That norepinephrine exerts a ketogenic effect independent of its lipolytic effect has been previously suggested by a study in diabetic man.[5] However, the results of that study are not directly comparable to those of the present study for two reasons. First, the dose of norepinephrine infused in the present study was half that used in the previous study in diabetic subjects. Second, all subjects in the present study had normal endogenous insulin secretion when measured following a 100-g glucose tolerance test to exclude diabetes.[22] The conclusion that norepinephrine exerts ketogenic activity independent of its lipolytic activity is based upon the assumption that the availability of free fatty acid to support hepatic ketogenesis was not different in the control-heparin study and the norepinephrine infusion study. Although this was statistically true when the plasma free fatty acid concentration during the two studies was assessed by comparing each observation point and by integrating the areas under the concentration curves, Figure 1 does suggest that the time course of the rise in plasma free fatty acid concentration was not identical in the two studies. Figure 1 suggests that plasma free fatty acid concentration peaked in the control-saline study before the maximal concentration in the norepinephrine infusion study. Since the hepatic conversion of fatty acids into ketone bodies requires about 10 min to be observed,[23] a more rapid increase in free fatty acid availability in the control-heparin study would actually favor increased rather than the observed relative decrease in ketosis in that study.

The conclusion that norepinephrine exerts ketogenic activity independent of its lipolytic activity also assumes that norepinephrine does not enhance the hepatic uptake of free fatty acids from plasma. Although no data are available in man, in the dog no effect of norepinephrine on hepatic FFA uptake was observed.[24] In contrast, in rat liver perfusion experiments, an actual catecholamine-induced decrease in hepatic FFA extraction has been observed, which was inhibited by dibenzyline.[25] Thus, in agreement with a previous study in diabetic man, our present study suggests that norepinephrine also exerts ketogenic activity in normal man independent of its lipolytic activity and at plasma hormone concentrations frequently observed in the plasma of man under stress.[1,8–10]

The mechanism by which norepinephrine exerts its ketogenic activity is not resolved by our study. As previously reviewed,[5] catecholamines have been shown to enhance ketogenesis in vitro in liver perfusion experiments[25] and in isolated hepatocytes.[26] However, in addition to a direct effect of norepinephrine on hepatic ketogenesis, norepinephrine might elevate plasma ketone body concentration either by decreasing the peripheral utilization of ketones or by decreasing the volume of distribution in which ketone bodies are present. Currently, no information is available concerning these latter two potential mechanisms.

In addition to a direct ketogenic effect of norepinephrine, this hormone may also induce ketosis by means of secondary hormonal effects. In the current study two possibilities are suggested. Firstly, norepinephrine suppressed plasma insulin concentration, which has known antiketogenic activity.[27] Secondly, norepinephrine also stimulated plasma glucagon concentration, which has been previously shown to possess ketogenic activity independent of its lipolytic effect.[28–30] Although norepinephrine-induced glucagon secretion may have contributed to the observed ketosis, additional studies utilizing somatostatin suppression of endogenous glucagon secretion will be necessary to resolve this possibility.

The results of this study emphasize the important role that endogenous insulin secretion has on modulating the catabolic effects of stress hormones. Since both glucagon and the catecholamines may exert their catabolic effects by stimulation of cyclic AMP,[29,31] it is of interest that, in vivo[23] but not in vitro,[31] these hormones exert a differential response on ketogenesis. The most attractive explanation for this observation is the differential effect of these hormones on endogenous insulin secretion. Thus, glucagon, which stimulates endogenous insulin secretion, exerts no net plasma ketogenic effect in vivo in man.[23] The present study demonstrates that norepinephrine infusion, which suppresses plasma insulin secretion, may induce a fourfold elevation in circulating plasma ketone body concentration within a 40-min period (Figure 1). Thus, the results of this study, in concert with a previous study,[5] firmly establish norepinephrine as a major ketogenic hormone in both normal and diabetic man.

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