Response of Glucose, Insulin, Free Fatty Acid, and Human Growth Hormone to Norepinephrine and Hemorrhage in Normal Man

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HYPERGLYCEMIA HAS been observed following infusion of epinephrine[13] and norepinephrine;[14] in acute blood loss;[17] and after battle injury.[3] The lack of an increase in the immunoreactive insulin levels while the glucose is elevated in these conditions is evidence that the persistence of hyperglycemia is due to catecholamine suppression of insulin release. Release of norepinephrine occurs after the loss of blood[20] and it has been suggested as an important mediator in the response to volume reduction.[19] The present study was made to simultaneously assess the metabolic and hemodynamic changes of isotonic volume reduction and catecholamine infusion.

Methods

Five healthy young men, 21 years of age, were hospitalized for 48 hours. A physical examination was done before admission. The risks of the study were explained and an informed consent obtained. The fasting subjects were placed in bed for measurement of their blood volume and cannulation. These procedures were required for the hemodynamic measurements and have been described in detail elsewhere.[7] A cannula in the radial artery was used for blood sampling and to remove blood during the hemorrhage period. A venous cannula placed in the superior vena cava was used for the infusion of norepinephrine. Both cannulae were in place for 36 hours and were kept patent by periodically flushing with heparinized saline solution (4 units/ml.).

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On the day of study, hemodynamic control values were established in the fasting subjects and blood was drawn for glucose, insulin, free fatty acids, and human growth hormone determinations. Norepinephrine (Levophed, bitartrate injection 0.2%, Winthrop Laboratories, N.Y., N.Y.) was then infused (Table 1), using a Holter pump (Extracorporeal Medical Specialties, Inc., Mt. Laurel, New Jersey), in three sequential doses ranging from 0.1 to 0.6 μg/K./min. (Table 2). The three doses were used to determine the hemodynamic dose response to norepinephrine, but blood samples were drawn only during infusion of the largest dose (0.42 to 0.60 μg K./min.). Sampling volumes of the first two periods were replaced, isovolumetrically, with blood from known donors and a recovery period of greater than one hour followed (Table 1). The samples were then repeated.

After an interval of 2 hours, hemorrhage was carried out in two stages (Table 2). The first stage was the sampling volumes from the recovery period, just completed, which was followed by rapid removal of blood from the radial arterial cannula in a quantity sufficient to total 15% of the subject's measured blood volume. After completion of the hemorrhage, samples were taken. The blood pressure had reached its lowest point and was rising at the time of sampling. Norepinephrine reinfusion followed immediately and the final samples were obtained to observe the metabolic response to the drug during hypovolemia. The experiment was then terminated and the blood volume restored. The men were observed after decannulation until the following

TABLE 1. *Experimental Periods in Individual Subjects Mean and Standard Deviation for Five Subjects*

Period	Minutes
C = control	103 + 26
N-1 = norepinephrine infusion	64 + 11
R = recovery	115 + 18
H = hemorrhage	
(a) = time for blood removal	16 ± 3
(b) = post-hemorrhage period	27 ± 5
N-2 = norepinephrine reinfusion	56 + 23

morning when they were discharged from the hospital.

Analytical Technics: Samples of plasma for glucose and serum for fatty acids, insulin, and growth hormone were frozen and stored until analysis. Whole blood samples for lactate were placed in 0.6M perchloric acid and centrifuged immediately. Lactate was measured using the U.V.-test (Boehringer, Mannheim Gmbht, Germany). The blood glucose was determined, employing a modification of the method of Hoffman,[9] which utilizes the potassium ferricyanide-potassium ferrocyanide oxidation reduction reaction. The measurement was made using the AutoAnalyzer (Technicon Instruments Corporation, Tarrytown, New York). One milliliter aliquots of serum were measured using the method of Dole and Meinertz,[5] extracting the sample with a two phase heptaneisopropyl alcohol water system to determine long chain nonesterified fatty acids.

Serum human growth hormone and insulin were determined together by using two labels, ^{131}I for insulin and ^{125}I for human growth hormone. Antibody was supplied by Arnel, N.Y., N.Y. and the double antibody technic modified from Yalow and Berson[22] by Soeldner and Slone[18] was used for separation of free and bound fractions. The tracer is supplied by Abbott Laboratories, North Chicago, Illinois and the standards are prepared from human growth hormone supplied by Dr. A. E. Wilhelmi, NIH GH HS 1216C and the insulin from human insulin 258 1025 B 88 (Eli Lilly, Indianapolis, Indiana). Data are presented as mean and standard deviation for the group of five subjects. Statistical significance is indicated in the Figures.

Results

Table 3 shows the values for plasma glucose, insulin, and lactate during the five periods of the study. The plasma glucose levels rose in all of the subjects with norepinephrine infusion from 96 ± 5 to 130 ± 12 mg./100 ml. This is shown graphically in Figure 1 and the change is highly significant. The insulin results are also shown in Figure 1 and no change is seen in response to the drug. After the recovery period, which followed cessation of the norepinephrine, the plasma glucose returned to control values, 99 ± 9 mg./100 ml. Insulin again remained unchanged in the group, but in Subject 5 the insulin was 21 microunits per ml. This subject also shows a persistent elevation of plasma glucose, 24 mg./100 ml. greater than the control values, suggesting that return to the resting state was not complete at the time of sampling. When the subsequent hemorrhage was complete, neither the glucose nor the insulin showed

TABLE 2. *Norepinephrine Dose and Hemorrhage Volume Removed in Individuals Mean and Standard Deviation for Five Subjects*

Norepinephrine (μg/Kg./min).	
Dose 1	.12 ± .02
Dose 2	.25 ± .04
Dose 3	.5 ± .07
Hemorrhage (ml.)	
Samples	175 ± 29
Rapid Arterial	727 ± 60
Per cent Total	15.3 ± .6

TABLE 3. *Response of Plasma Glucose, Immunoreactive Insulin and Blood Lactate to Norepinephrine and Hemorrhage*

Subject / Period	1	2	3	4	5	Mean and Standard Deviation
			Glucose (mg./100 ml.)			
C	95	97	102	98	89	96 ± 5
N-1	131	112	146	131	128	130 ± 12
R	93	91	102	98	113	99 ± 9
H	107	94	94	90	102	97 ± 7
N-2	137	126	122	127	161	135 ± 16
			Insulin (uU/L)			
C	10	15	11	10	10	11 ± 2
N-1	10	13	10	15	11	12 ± 2
R	10	10	10	11	21	12 ± 5
H	10	10	10	10	14	11 ± 2
N-2	10	26	10	10	15	14 ± 7
			Lactate (mM/L)			
C	0.5	0.5	0.4	0.5	0.5	0.5 ± 0.0
N-1	0.8	0.7	0.5	0.7	0.9	0.7 ± 0.2
R	0.5	0.4	0.7	0.6	1.2	0.7 ± 0.3
H	0.9	0.5	0.6	0.5	0.8	0.7 ± 0.2
N-2	0.9	0.5	0.6	0.6	1.1	0.7 ± 0.3

C = control; N-1 = norepinephrine infusion; R = recovery; H = hemorrhage; N-2 = norepinephrine reinfusion.

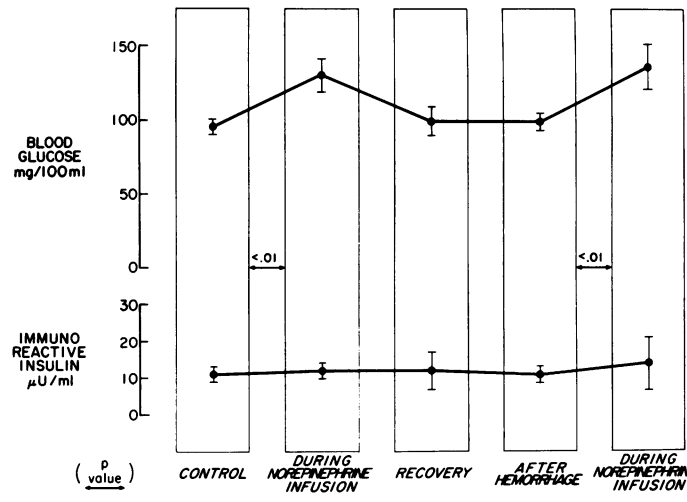

FIG. 1. Response of plasma glucose and immunoreactive insulin in five normal men to norepinephrine infusion and hemorrhage. The mean and standard deviation are presented. n = 5. Statistical significance is determined by the Student's *t* test.

changes (Fig. 1), but again one individual, Subject 1, had an elevation of glucose above normal, 107 mg./100 ml. When norepinephrine infusion was repeated during the hypovolemic state the response was similar to that seen with the drug infusion prior to hemorrhage: glucose rose and insulin remained unchanged. The statistical significance is shown in Figure 1.

Free fatty acids are given for only three of the subjects during the control period (Table 4). A rise was seen with norpinephrine from 1390 ± 290 to 3000 ± 820 μEq./l, (Fig 2). After recovery the control level was re-established, 1420 ± 560 μEq/l, but the response to hemorrhage was equivocal and the group showed no significant change. The final rise with norepinephrine reinfusion from 1640 ± 655 to 2470 ± 570 μEq./liter was statistically significant $p < 0.01$ (paired t).

Human growth hormone values for the individual subjects are shown in Table 5 and the group response in Figure 3. Values are reported for three subjects during the control period. With norepinephrine infusion the growth hormone fell from 5.6 ± 3.6 to 0.9 ± 0.5 ng./ml, $p < 0.05$ (Student's t test). During the recovery period the growth hormone did not return to control levels (Fig. 3) but remained depressed. After hemorrhage the values for growth hormone rose from 0.7 ± 1.0 to 4.2 ± 2.8 ng./ml. Norepinephrine reinfusion again showed a significant drop to 0.8 ± 0.4 ng./ml., $p < 0.05$ (Student's t test). The consistent response to norepinephrine is shown in Figure 3 during both the infusion and the post-hemorrhage reinfusion periods.

Discussion

Resting values of glucose were high for the fasting subjects of this study, as were the values for free fatty acids and growth hormone, indicating that the control period was not a true resting basal state. The men were not conditioned and the experiment was a new experience which may account for the increased sympathoadrenal activity. Norepinephrine infusion caused a further glycogenolysis which resulted in hyperglycemia. This metabolic response to norepinephrine has not been as extensively studied as the response to epinephrine, but the results of this study indicate that norepinephrine causes changes in the glucose and insulin relation similar to those which other investigators have shown with epinephrine.[10,13] Porte and Williams[14] have also studied normal subjects and have shown that immunoreactive insulin concentration was less than expected during norepinephrine infusion, 6 μg./min. They concluded that insulin secretion was inhibited, but the effect was quantitatively less than that obtained using an equivalent dose of epinephrine. In the present study larger norepinephrine doses were used, but the hyperglycemia reached levels similar to those reported by Porte and Williams[14] and the increase in insulin secretion was completely suppressed.

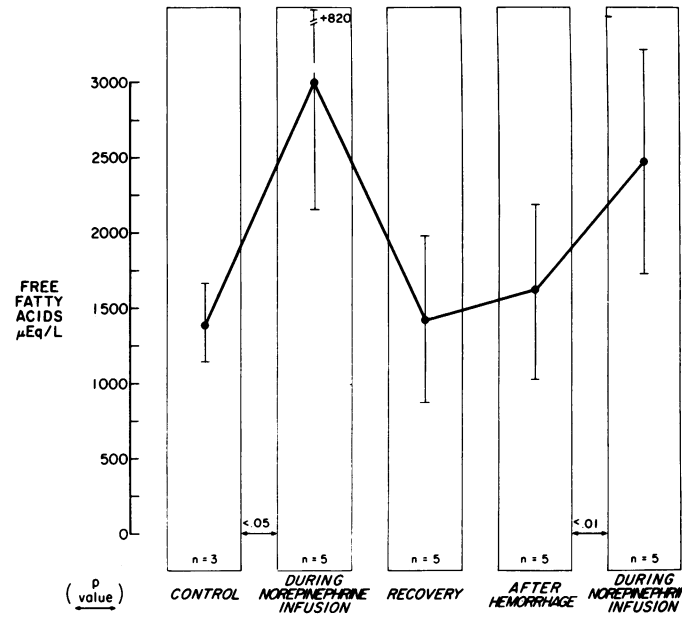

FIG. 2. Response of free fatty acids in five normal men to norepinephrine and hemorrhage. The mean and standard deviation are represented. n = 5 for all periods except the control for which n = 3. Statistical significance is determined by the paired t test.

TABLE 4. Response of Free Fatty Acids to Norepinephrine and Hemorrhage

Subject	1	2	3	4	5	Mean and Standard Deviation
	Free Fatty Acids (μEq/L)					
Period						
C	1185	1720	—	—	1270	1390 ± 290
N-1	2970	3610	3885	2720	1790	3000 ± 820
R	1500	1390	2250	1280	690	1420 ± 560
H	1720	2500	1945	1220	805	1640 ± 655
N-2	2060	3215	2795	2470	1790	2470 ± 570

C = control; N-1 = norepinephrine infusion; R = recovery; H = hemorrhage; N-2 = norepinephrine reinfusion

TABLE 5. Response of Human Growth Hormone to Norepinephrine and Hemorrhage

Subject	1	2	3	4	5	Mean and Standard Deviation
	Human Growth Hormone (NG/ml.)					
Period						
C	9.2	5.5	—	—	2.0	5.6 ± 3.6
N-1	1.5	0.6	0.2	1.1	0.9	0.9 ± 0.5
R	0.4	2.6	0.2	0.2	0.3	0.7 ± 1.0
H	7.0	2.4	0.3	5.4	6.1	4.2 ± 2.8
N-2	1.2	0.4	1.2	0.5	0.6	0.8 ± 0.4

C = control; N-1 = norepinephrine infusion; R = recovery; H = hemorrhage; N-2 = norepinephrine reinfusion

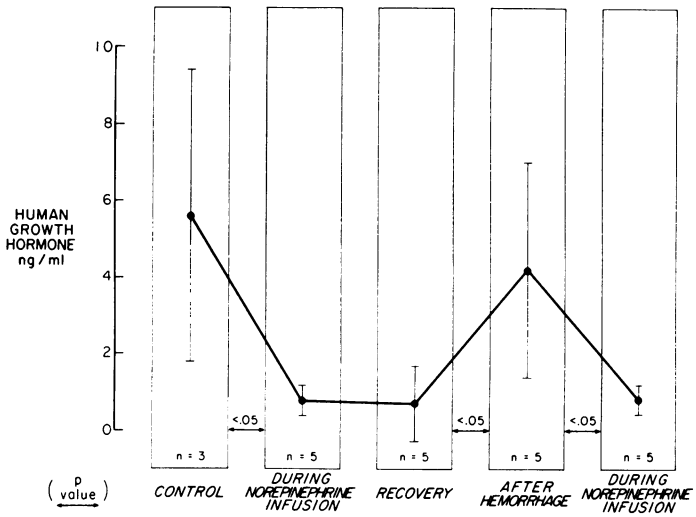

FIG. 3. Response of human growth hormone in five normal men to norepinephrine and hemorrhage. The mean and standard deviation are represented. n = 5 for all periods except the control for which n = 3. Statistical significance is determined by the Student's *t* test.

It is generally accepted that the catecholamine-induced hyperglycemia is the result of glycogenolysis. The absence of an elevated blood lactate is evidence that liver glycogen is making the major contribution to the elevated blood sugar and not muscle glycogenolysis to lactate recycling to glucose via the liver.[6] The hyperglycemia has been shown to persist as long as the catecholamine is infused, up to 7 hours with epinephrine,[13] and falls only when the drug is stopped.

The concomitant suppression of blood insulin relative to glucose level is very clearly shown in this study and is consistent with a direct effect of the catecholamine upon the beta cell of the pancreas. Decreased islet flow and increased hepatic extraction of insulin have also been suggested but direct effect upon the cell is favored.[11] The implication is that once the blood sugar rises it remains elevated as a result of suppressed insulin secretion and continued glucose production. This is supported by the fact that cessation of infusion causes a prompt rise in insulin and a decrease in blood glucose.[14]

With the hemorrhage alone there was no elevation of blood glucose. This amount of blood loss had been sufficient to show an increase in peripheral vascular resistance,[7] indicating that the blood loss was enough to activate the sympathetic nervous response, but was not enough to cause detectable hepatic glycogenolysis. Skillman, Hedley-White and Pallota[17] have shown a rise in blood glucose with this degree of hemorrhage in man, but Carey and Wallack[4] have shown in animals that the volume and rate of blood removal are important factors in the response of the blood glucose, and they saw no hyperglycemia after a slow 30% hemorrhage. An important difference between the studies of Skillman *et al.* and this study may have been in the rate and manner of bleeding as well as the use of untrained subjects who started, in our study, with evidence of increased sympathetic activity as evidenced by slightly elevated glucose and free fatty acid levels.

The infusion of norepinephrine before hemorrhage may also have been an attenuating factor, but full recovery was permitted before the blood removal and no residual effect would be anticipated this long after the drug.[21] Reinfusion of norepinephrine during hypovolemia caused the same hyperglycemia with insulin suppression which had been seen before hemorrhage, showing that sufficient glycogen stores remained after the first stimulus and when adequately rechallenged could respond to the same degree.

The mobilization of plasma free fatty acids in response to norepinephrine infusion has been studied by Havel and Goldfein in dogs.[8] These authors have concluded that stimulation of the sympathetic nervous system causes the release of free fatty acids from adipose tissue. Their observations in humans in whom plasma free fatty acids rose in response to anxiety and discomfort is further evidence that the sympathetic nervous system mediates this response. Havel and Goldfein also measured blood glucose in their human subjects, but did not show the rise which was seen in the subjects of the present study. The drug was administered in a smaller dose over a shorter period of time in the earlier study (100 μg./min.) which may account for the failure of blood glucose to rise and suggests that the hyperglycemia is a later response than the rise in fatty acids when norepinephrine is infused. This possibility is not substantiated by the present study, but there is evidence that blood glucose and free fatty acids both rise after norepinephrine infusion for 1 hour. The control of human growth hormone has become a controversial topic, but a number of studies have shown that catecholamines may be activators of its release.[2,16] This topic has been confused by the fact that hyperglycemia causes a decrease in plasma growth hormone.[1] When norepinephrine was given to the subjects of this study, the growth hormone values fell as a result of the hyperglycemia, indicating that this effect is stronger than adrenergic stimulation. Muller, Pra, and Pecile[12] have emphasized the point that the blood-brain barrier is impermeable to the catecholamines and have demonstrated in rats that growth hormone can be released by direct intraventricular injections of epinephrine, norepinephrine, and dopamine. The findings of this study do not illuminate the primary effect of norepinephrine upon growth hormone secretion, but do indicate that its effect is to raise the blood sugar and thereby to decrease the growth hormone secretion.

Following hemorrhage, human growth hormone levels increased, but this probably represented a restoration of the control values after the hyperglycemia of the norepinephrine period rather than a direct effect of the hemorrhage.

Rabinowitz, Merimee, Burgess, and Riggs[15] have studied this problem in healthy females using epinephrine and have shown that both insulin and growth hormone are suppressed during the epinephrine-induced hyperglycemia. However, addition of arginine to the infusion overcame the effects of epinephrine and both insulin and growth hormone rose despite continued epinephrine infusion, illustrating that the pathways for growth hormone and insulin suppression are not unique.

Summary

The metabolic response during norepinephrine infusion and hemorrhage was studied in five male volunteers. Fasting blood samples were obtained in the resting state for measurement of blood glucose, serum insulin, blood lactate, serum free fatty acids, and serum growth hormone. A norepinephrine infusion was given and the measurements were repeated. After recovery the men were bled by arterial hemorrhage of 15 per cent of their measured blood volume. Following sampling, norepinephrine was reinfused during hypovolemia. A significant rise in blood glucose was shown during norepinephrine infusion before and after hemorrhage, but not with hemorrhage alone. Blood lactate did not rise and there was no insulin response despite the hyperglycemia. Free fatty acid levels rose during norepinephrine infusion and growth hormone declined. There was no response of any of the variables with the hemorrhage. The results of this study show that hyperglycemia following norepinephrine infusion is unaccompanied by a rise in insulin and growth hormone but there is evidence for free fatty acid mobilization. The hemorrhage which had caused a rise in peripheral vascular resistance was insufficient to cause a sympathoadrenal metabolic response.

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